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11993 P

Comparative Therapeutic Efficiency of Sulfapyridine and Sulfathiazole in Mice Infected with Pneumococcus Types II and III.

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Sulfathiazole, a thiazole derivative of sulfanilamide, was studied by Fosbinder and Walter,[1] as well as Lott and Bergeim.[2] The toxicity was subsequently investigated by VanDyke, *et al.*,[3] who found

1 Fosbinder, R. J., and Walter, L. A., *J. Am. Chem. Soc.*, 1939, **61**, 2032.

2 Lott, W. A., and Bergeim, F. H., *J. Am. Chem. Soc.*, 1939, **61**, 3593.

3 Van Dyke, H. B., Greep, R. O., Rake, G., and McKee, C. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 410.

repeated administrations of sulfathiazole or sulfapyridine in 2% concentration in the food of mice an indication that sulfathiazole is more toxic than sulfapyridine. If, however, 1% concentrations of the drugs were fed to mice, there was no difference in the toxicity. They found sulfapyridine more toxic for monkeys; sulfathiazole fed for 14 to 21 days caused a negligible loss of weight, while sulfapyridine feeding resulted in greater weight loss. Long, *et al.*,[4] found that the acute toxicity of sulfathiazole (as measured by the parenteral injection of the sodium salt) for mice is one-third greater than that of sulfanilamide, and about half that of sulfapyridine. McKee, *et al.*,[5] found that the therapeutic effects of sulfathiazole and sulfapyridine are equal in mice infected with various types of pneumococci and treated by administering the drugs in 1% diet. Barlow and Homburger[6] reported that the chemotherapeutic effect of sulfathiazole in mice with pneumococcus infection is definitely superior to sulfanilamide, and compares very favorably with sulfapyridine.

Chemically, sulfathiazole is 2-(p-aminobenzene sulfonamido) thiazole; it is sparingly soluble in water at room temperature, more soluble in boiling water from which it can be recrystallized. In the present investigation the compound used was prepared in the authors' laboratories and melted at 198-199°. Analysis showed nitrogen, 16.38%, theory 16.47%. Additional sulfathiazole purchased from commercial houses, which had the same chemical properties, was also included in this study.

Sulfathiazole is tolerated by rabbits *per os* in a dose of 3 to 5 g per kg of body weight, while the maximum tolerated dose of sulfapyridine, as reported by us previously,[7] is 2 g per kilo. Mice receiving sulfathiazole in aqueous suspension *per os* tolerated 10 to 15 g per kilo, while sulfapyridine was tolerated in a dose of 20 g per kilo.

Therapeutic Effect. The mice used in this study were infected intraperitoneally with 20 to 200 minimum lethal doses of types II or III pneumococcus of which the average minimum lethal dose was 0.5 cc of 1:10,000,000 dilution of broth culture. The culture was

[4] Long, P. H., Haviland, J. W., and Edwards, L. B., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 238.

[5] McKee, C. M., Rake, G., Greep, R. O., and Van Dyke, H. B., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 417.

[6] Barlow, O. W., and Homburger, E., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 317.

[7] Raiziss, G. W., Severac, M., Moetsch, J. C., and Clemence, L. W., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 434.

TABLE I.
Comparative Therapeutic Effects of Sulfapyridine and Sulfathiazole Administered by Mouth in Types II and III Pneumococcic Infection in Mice.

Type of pneumococcus	Drug	No. mice used	% of survivals in days									
			1	2	3	4	5	6	7	14	21	28
II	Sulfapyridine	40	100	100	93	85	65	50	43	28	28	28*
	Sulfathiazole	60	100	90	82	73	52	25	13	5	3	3
	Controls	58	5	0								
III	Sulfapyridine	26	100	100	85	77	65	54	38	12	12	12
	Sulfathiazole	33	94	82	58	27	18	15	6	3	3	3
	Controls	25	48	48	8	0						

*In our earlier work[7] we reported a smaller percentage of survivals following sulfapyridine treatment than we do in the present investigation. The difference in results is due to the fact that in our previous work the first dose of sulfapyridine was administered one and one-half hours after infection, and only two doses per day were given the first two days. In the present work we treated the mice immediately after infection, and have given three doses per day for five consecutive days. The maximal number of treatments in our earlier work was ten; in the present experiments eighteen doses were administered.

prepared as follows: Mice were infected intraperitoneally with 1 to 1.5 cc of the straight culture. When the mouse died (usually within 6 to 8 hours) it was autopsied and a loop full of heart blood was inoculated into the blood broth media. This culture was ready for use in 14 to 16 hours.

The drugs were given by mouth in a dose of 10 mg. The mice were dosed three times daily, at 9 a.m., 5 p.m., and 12 m, for 5 days, at 9 a.m. and 5 p.m. on the sixth day, and at 9 a.m. on the seventh day—a maximum of 18 treatments. Table I presents a summary of the results obtained in several experiments.

Summary. Studies of the toxicity disclose that sulfathiazole administered *per os* to rabbits is less toxic than sulfapyridine. On the other hand, administered the same way to mice sulfapyridine appeared to be less toxic than sulfathiazole. It is obvious that the absorption and elimination, and the resulting toxicity of the drug depend upon the species of animal used. In Types II and III pneumococcus infection, the therapeutic effect of sulfapyridine is superior to that of sulfathiazole, based on the oral dose only and with no blood level determinations.

11994

Comparison of Antistreptococcal Activities of p-Caproylaminobenzenesulfonhydroxamide and Sulfanilamide.

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The recent appearance of a number of papers[1-4] dealing with various aspects of the chemotherapeutic activity of p-caproylaminobenzenesulfonhydroxamide indicates an increasing interest in this compound. To facilitate further investigation, we report here the results of a comparative study of this sulfonhydroxamide and p-aminobenzenesulfonamide in the treatment of streptococcal infections in mice.

Methods and Materials. 1. *Chemotherapeutic Experiments.* Hemolytic streptococci (strain 1896) were grown for 6 hours in meat infusion broth containing 5% rabbit blood. The culture was diluted to permit the administration of 0.5 cc in each intraperitoneal injection. Dilutions of 10^{-3}, 10^{-4}, and 10^{-5}, approximately equal to 10,000, 1000, and 100 lethal doses respectively, were used. Virulence was maintained by frequent mouse passage.

The drugs were pulverized and suspended in 10% gum acacia. Each mouse received 0.5 cc of the appropriately diluted suspension by stomach tube at the intervals stated.

Mice weighing 18 to 20 g were inoculated intraperitoneally with 0.5 cc of the diluted streptococcus culture. Five or 10 mg doses of the drugs were fed to the mice immediately after infection, and then once daily for 5 additional days. Observations were made over a period of 10 days. The results are summarized in Table I. Under these conditions p-caproylaminobenzenesulfonhydroxamide was somewhat more effective than the sulfanilamide which was used for comparison.

A second series of infected mice were given 2 mg doses of the sulfonhydroxamide at 4-hour intervals over a period of 96 hours. Single daily doses were then given for 3 additional days. For compari-

[1] Cooper, F. B., Gross, P., and Lewis, M., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 491.

[2] Main, E. R., Shinn, L. E., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 593.

[3] Kohl, M. F. F., and Flynn, L. M., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 455.

[4] Shinn, L. E., Main, E. R., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 596.

TABLE I.

Materials—Six-hour culture *Streptococcus hemolyticus* 1896. Dilution of culture, 10^{-3}, 10^{-4}, 10^{-5} equivalent to 10,000, 1,000 and 100 lethal doses respectively.

Method—Mice were infected intraperitoneally with 0.5 cc of culture, and fed immediately after the injection and once daily for 5 additional days.

No. of mice	Compound	Dose per mouse in mg	Dilution of culture	% survival Time in days								
				1	2	3	4	5	6	7	8	9
45	Sulfanilamide	5	10^{-3}	73	33	24	24	24	24	24	24	24
45	The sulfonhydroxamide	5	10^{-3}	88	49	44	38	38	38	35	35	35
45	Sulfanilamide	10	10^{-3}	88	40	33	33	33	33	33	24	22
45	The sulfonhydroxamide	10	10^{-3}	91	67	51	49	47	47	47	44	44
45	Sulfanilamide	5	10^{-4}	93	69	60	58	56	51	49	47	47
45	The sulfonhydroxamide	5	10^{-4}	98	80	69	67	64	64	64	60	58
45	Sulfanilamide	10	10^{-4}	96	73	71	67	67	67	64	62	58
45	The sulfonhydroxamide	10	10^{-4}	93	84	80	80	78	78	76	73	71
45	Sulfanilamide	5	10^{-5}	93	71	69	67	64	64	64	62	62
45	The sulfonhydroxamide	5	10^{-5}	96	82	82	82	82	82	76	73	71
45	Sulfanilamide	10	10^{-5}	100	84	82	82	82	78	73	73	73
45	The sulfonhydroxamide	10	10^{-5}	100	98	98	98	93	91	89	89	89
15	Controls	—	10^{-4}	13	0	0	0	0	0	0	0	0
30	"	—	10^{-5}	24	0	0	0	0	0	0	0	0

son, similar experiments were performed using equal-weight doses of sulfanilamide as the chemotherapeutic agent. Observations were made each time the drugs were administered, but in the interests of space a limited number of observations are shown in Table II

At culture dilutions of 10^{-4} and 10^{-5} no significant differences between the two drugs were observed. At a dilution of 10^{-3} sulfanilamide gave a greater protection than the sulfonhydroxamide during the 4-day period in which the drugs were administered at 4-hourly intervals. This greater effectiveness probably results from the higher average blood level maintained with sulfanilamide (Fig. 2) rather than from a higher chemotherapeutic activity (at equal blood levels). When the surviving animals were given single daily doses of the drugs, the differences observed at this culture dilution disappeared.

2. *Colorimetric Study of Blood Concentrations.* Sulfanilamide determinations were performed by the method of Bratton and Marshall.[5] For the estimation of p-caproylaminobenzenesulfonhydroxamide and its deacylated analogue the following method was devised:

To 1 cc of blood add 19 cc of 95% alcohol, shake, allow to stand for 15 minutes and filter. To 5 cc of the filtrate add 1 cc of 4 N-H_2SO_4 and heat for 75 minutes on a water bath. Cool and

[5] Bratton, A. C., and Marshall, E. K., Jr., *J. Biol. Chem.*, 1939, **128**, 537.

TABLE II.

Materials—A 6-hour culture of streptococcus (1685) equivalent to 1,000,000, 100,000, and 10,000 lethal doses.

Method—Mice were infected intraperitoneally with 0.5 cc of diluted culture and treated orally with 2 mg of sulfanilamide or the sulfonhydroxamide immediately after infection and then at 4-hour intervals for 96 hours. Twelve mg doses were then given daily for 3 additional days.

No. of mice	Drug	Dilution of culture	No. of animals surviving Time in hr								
			24	36	48	60	96	120	144	168	192
15	Amide	10^{-5}	15	15	15	15	15	15	14	14	14
15	Hydroxamide	10^{-5}	15	15	15	15	15	15	14	12	12
15	Amide	10^{-4}	15	15	15	15	13	13	10	8	7
15	Hydroxamide	10^{-4}	15	15	15	14	14	14	11	7	7
15	Amide	10^{-3}	15	15	14	13	9	8	2	1	1
15	Hydroxamide	10^{-3}	15	13	12	10	5	5	3	0	0
5	Controls	10^{-3}	0	0	0	0	0	0	0	0	0
5	"	10^{-4}	0	0	0	0	0	0	0	0	0
5	"	10^{-5}	1	0	0	0	0	0	0	0	0
2	"	10^{-6}	1	0	0	0	0	0	0	0	0
2	"	10^{-7}	2	0	0	0	0	0	0	0	0
2	"	10^{-8}	2	1	0	0	0	0	0	0	0
2	"	10^{-9}	2	2	0	0	0	0	0	0	0
2	"	10^{-10}	2	2	1	1	1	1	1	1	1

Amide—Sulfanilamide.
Hyroxamide—p-Caproylaminobenzenesulfonhydroxamide.

adjust the volume to 15 cc and filter.* The diazotization and coupling are then carried out essentially as in Marshall's procedure. To 10 cc of the filtrate add 1 cc of 0.1% sodium nitrite and mix. After 3 minutes add 1 cc of 0.5% ammonium sulfamate and mix. After 3 minutes add 1 cc of 0.1% N-(1-naphthyl)ethylene diamine dihydrochloride and mix. After 5 minutes add 5 cc of alcohol† and read in the Evelyn colorimeter using filter No. 540. This gives the "total" p-caproylaminobenzenesulfonhydroxamide. The "free" deacylated form is determined by evaporating 5 cc (or 10 cc if the concentration is low) of the original alcoholic filtrate to dryness. Cool, and add 1 cc of 4 N-H_2SO_4 and water to 15 cc. Filter and diazotize 10 cc of this filtrate as described above.

Several series of recovery experiments were performed in which the drug, its deacylated free base, and a mixture of both substances were added to whole blood. An average recovery of 96% (±4)

* Diazotization and coupling cannot be satisfactorily performed in the presence of alcohol due to an alteration of reaction rates which permits nitrosation of the beta component. This is prevented by evaporating off the alcohol.

† This dissolves lipoid material to give an optically clear solution.

was obtained in 30 such experiments. Final values are, therefore, divided by 0.96.

Results of the blood determinations in mice are shown in Figs. 1 and 2. Each point was established by analyzing the pooled blood of 5 mice. Two or more groups of 5 were used for each determination. Following the oral administration of single doses of the drug, blood levels of the free and total sulfonhydroxamide were remarkably constant indicating uniform absorption. Only slight differences in the blood levels were obtained following 5 or 10 mg doses, probably indicating a limited absorption. In equal doses, sulfanilamide affords much higher blood concentrations.

When the drugs were fed at 4-hour intervals, the blood levels of the sulfonhydroxamide were much lower than those obtained with sulfanilamide. However, as shown in Fig. 2, the level was main-

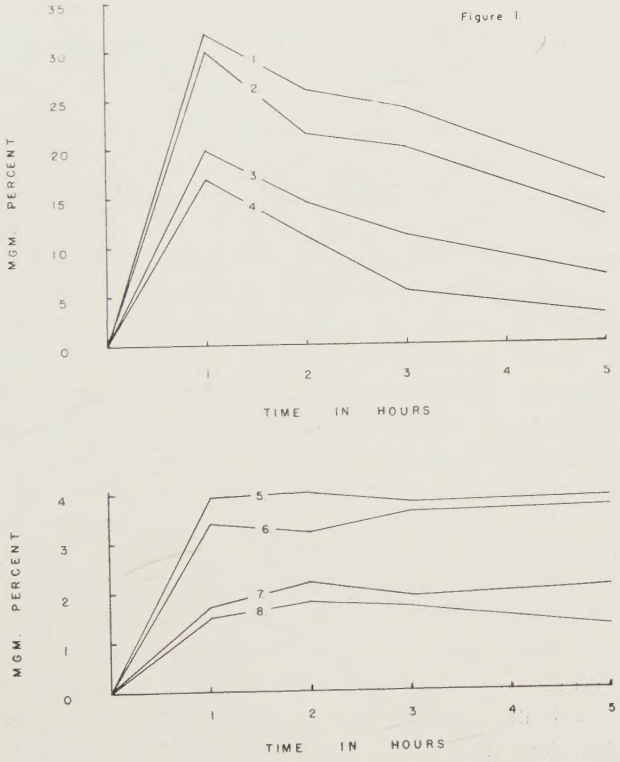

FIG. 1.

Blood Concentrations Following a Single Oral Dose of the Drugs.
Curves 1 and 2: Total and free sulfanilamide following 10 mg dose.
Curves 3 and 4: Total and free sulfanilamide following 5 mg dose.
Curves 5 and 7: Total and free sulfonhydroxamide following 10 mg dose.
Curves 6 and 8: Total and free sulfonhydroxamide following 5 mg dose.

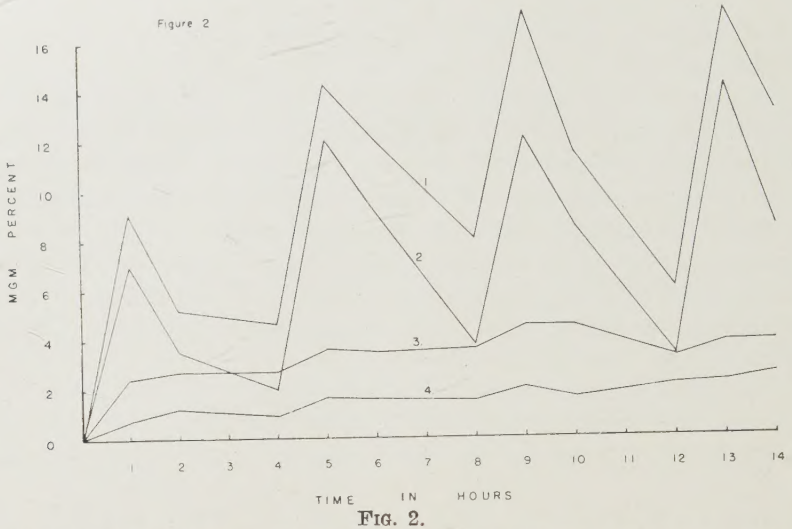

FIG. 2.

Blood concentrations following 2 mg doses of the drugs administered every 4 hours. Curves 1 and 2 represent the total and free sulfanilamide. Curves 3 and 4 represent the total and free sulfonhydroxamide, respectively.

tained much more steadily with the sulfonhydroxamide than with sulfanilamide.

Discussion. Recently Shinn, Main, and Mellon[4] demonstrated an immediate, although transient, bacteriostasis due to the sulfonhydroxamide group of p-caproylaminobenzenesulfonhydroxamide which was followed by a secondary effect due, presumably, to hydrolysis of the caproyl group. Such a mechanism suggests that the sulfonhydroxamide might differ from sulfanilamide in antistreptococcal activity. In agreement with the data of Cooper, Gross, and Lewis[1] we have found no striking differences in the activity of sulfanilamide and the sulfonhydroxamide when the drugs were given every 4 hours by mouth. However, the sulfonhydroxamide produced this effectiveness at lower blood concentrations than sulfanilamide. This difference is even further accentuated by comparison of the blood levels on a molecular basis, since one molecule of the sulfonhydroxamide is equivalent to 1.6 molecule of sulfanilamide.

Since for an optimal therapeutic effect the maintenance of an effective blood concentration is essential,[6] a drug which, because of solubility and other properties, is maintained at constant levels in the blood is desirable. Fig. 2 shows that more constant levels are maintained with the sulfonhydroxamide than with sulfanilamide. This may explain why sulfanilamide and the sulfonhydroxamide

[6] Marshall, E. K., Jr., Litchfield, J. T., Jr., and White, H. J., *J. Pharm. and Exp. Therap.*, 1940, **69**, 89.

are approximately equally effective when fed at intervals of 4 hours, whereas the sulfonhydroxamide is the more effective when the drugs are fed once daily.

Summary. 1. A colorimetric method for the determination of p-caproylaminobenzenesulfonhydroxamide has been described. 2. This drug is approximately equal, weight for weight, to sulfanilamide in antistreptococcal activity when fed at intervals of 4 hours, but it is somewhat more effective than sulfanilamide when fed only once daily. 3. When the sulfonhydroxamide and sulfanilamide were given in equal-weight oral dosage, the former gives the lower and more constant blood levels.

11995

Inhibition by Sulfapyridine of the Curative Action of Nicotinic Acid in Dogs.

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When *Staphylococcus aureus* is inoculated in a medium deficient in nicotinic acid, the addition of nicotinic acid will support growth, but the addition of nicotinic acid and sulfapyridine will not do so. The addition of coenzymes and sulfapyridine, however, gives excellent growth.[1] Dorfman[2] has shown that sulfapyridine inhibits the increased respiration of dysentery bacilli caused by nicotinic acid amide. The present investigation was undertaken to see if a similar relationship could be demonstrated in dogs on a diet deficient in nicotinic acid.

Young dogs were fed such a diet[3] until weight loss and diarrhea developed, although typical tongue lesions did not appear. Drugs and vitamins were administered orally in gelatin-coated capsules. Sodium sulfapyridine was given at 9 a. m. and 5 p. m. in doses sufficient to maintain a blood level of 3 to 10 mg per 100 cc just before the morning dose. Nicotinic acid was given in doses of

1 *a.* West, R., and Coburn, A. F., *J. Exp. Med.*, 1940, **72**, 91; *b.* West, R., and Coburn, A. F., *Tr. A. Am. Physicians*, 1940, 173.

2 Dorfman, A., Rice, L., Koser, S. A., and Saunders, F., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 750.

3 Koehn, C. J., Jr., and Elvehjem, C. A., *J. Biol. Chem.*, 1937, **118**, 693.

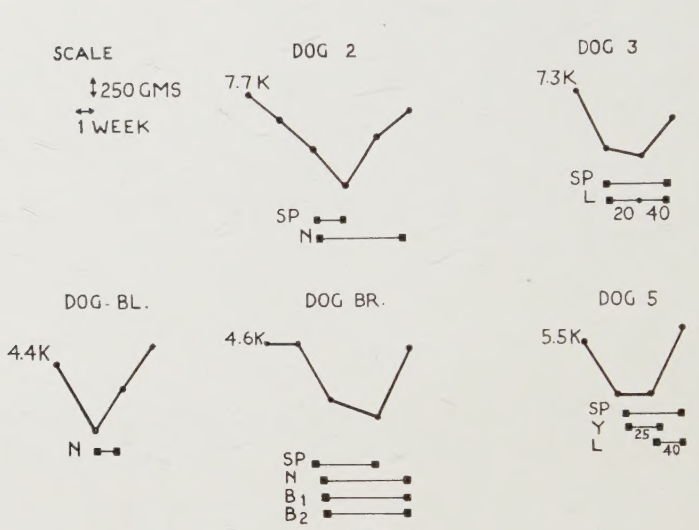

FIG. 1.

15 mg daily, thiamin chloride and riboflavin 0.2 mg each every second day.

The results are shown in the figure. Dog BL responded promptly to nicotinic acid, while dog 2 continued to lose weight when sulfapyridine was given with the nicotinic acid, but gained when the sulfapyridine was stopped and the nicotinic acid continued. Two other dogs, not shown in the figure, acted in a similar way, one of them dying before sulfapyridine was stopped. Dog BR received an additional supplement of thiamin and riboflavin and acted as dog 2 had. In all instances the sulfapyridine was started a day before the nicotinic acid.

As purified coenzymes were not available in sufficient amounts for this experiment, liver and brewers' yeast were fed in addition to the deficient diet. Dogs 3 and 5, though getting sufficient sulfapyridine to maintain a suitable blood level, gained weight when 40 g of raw beef liver were given daily, though 20 g of liver or 25 g of yeast had little effect. The liver was fed separately, but during the period of liver feeding greater amounts of the deficient diet were consumed, though sulfapyridine was given simultaneously. This makes it seem improbable that the weight lost during the sulfapyridine and nicotinic acid period was merely due to anorexia induced by the sulfapyridine.

Summary. When dogs were placed on a diet deficient in nicotinic acid, the addition of nicotinic acid corrected the deficiency, while the addition of nicotinic acid and sulfapyridine failed to do so. When raw liver and sulfapyridine were added to the diet, the deficiency disappeared. It seems possible, but not proven, that sulfapyridine inhibits the action of nicotinic acid but not of preformed coenzymes.

11996

Two Outbreaks of Influenza Caused by Antigenically Different Viruses.

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Early studies on influenza suggested that the virus agents of the disease, although not antigenically identical were closely enough related for the infection to evoke a demonstrable rise in antibodies reactive against the recognized strains of the influenza virus. The first convincing indication that influenza might be due to antigenically unrelated viruses was presented by Stuart-Harris, Smith and Andrewes,[1] who found that a rise in antibodies against the usual strains of influenza virus occurred in only 33% of the cases they studied in England in 1939; although they failed to isolate the actual virus, they concluded that a number of their cases must have been caused by an agent other than the usual influenza virus. More direct evidence has recently been obtained independently by Francis and by ourselves. In February, 1940, we[2] isolated a virus (termed *TM*) from 2 cases of influenza and showed that in each instance the infection evoked an increase in antibodies against the homologous *(TM)* virus but caused no detectable increase in antibodies reactive against the *PR8*[3] strain of influenza virus. At about the same time Francis[4] isolated a strain (termed "Lee") which was also distinct from the *PR8* and from other previously recognized strains. He showed that the con-

[1] Stuart-Harris, C. H., Smith, W., and Andrewes, C. H., *Lancet*, 1940, **1**, 205.

[2] Magill, T. P., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 162.

[3] Francis, T., Jr., *Science*, 1934, **80**, 457.

[4] Francis, T., Jr., *Science*, 1940, **92**, 405.

valescent serums from cases of influenza which had occurred in various places during the early part of 1940 and also serums from 2 cases that had occurred in 1936 had increased titers of antibodies against his *Lee* strain but not against the *PR8* strain. Francis concluded that his *Lee* strain was a new type of virus and proposed that it be termed *influenza B* to distinguish it from the older strains (PR8, WS, etc.) for which the term *influenza A* had already been suggested.[5] The relationship between Francis' *Lee* strain and our *TM* strain has not yet been determined, but since both were isolated in New York City at about the same time it is entirely possible that they are similar, if not identical. Although it is desirable that the serological properties of the two strains be compared in a future study, the important point is that the studies already made with each of the viruses have established that typical influenza can be caused by virus agents which are antigenically different than those previously described.

The present paper presents additional data in support of the general principle that clinically similar cases of influenza may be caused by viruses that are antigenically distinct. In the investigation 2 viruses were utilized: the *PR8* strain represents the older group of strains of influenza virus (*"influenza A"*), whereas the *TM* represents a virus which is antigenically distinct from the older group of strains. The 2 viruses were tested against serums obtained from cases of influenza during 2 outbreaks of the disease which occurred about a year apart (January, 1939, and February, 1940) among the nurses in The New York Hospital. All of the cases were hospitalized throughout the course of illness and hence the opportunities for clinical observation were especially satisfactory. It is important that even under these satisfactory conditions for study no consistent differences of a clinical sort were evident between the 2 groups of cases. Two samples of serum were obtained from each patient: the first within 24 hours of the time of appearance of symptoms, and the second 3 to 4 weeks later. Each pair of serums was tested against the 2 viruses; in the case of the *PR8* both protection tests and complement fixation tests were made, but in the case of the *TM* only protection tests were included because we had not yet developed a satisfactory complement fixation procedure for that virus. The results are summarized in Figs. 1 and 2 in which, for each virus, the titer of the serum obtained during the first 24 hours of illness is plotted against the titer of the serum obtained from the same person

[5] Horsfall, F. L., Lennette, E. H., Rickard, E. R., Andrewes, C. H., Smith, W., and Stuart-Harris, C. H., *Lancet*, 1940, **2**, 413.

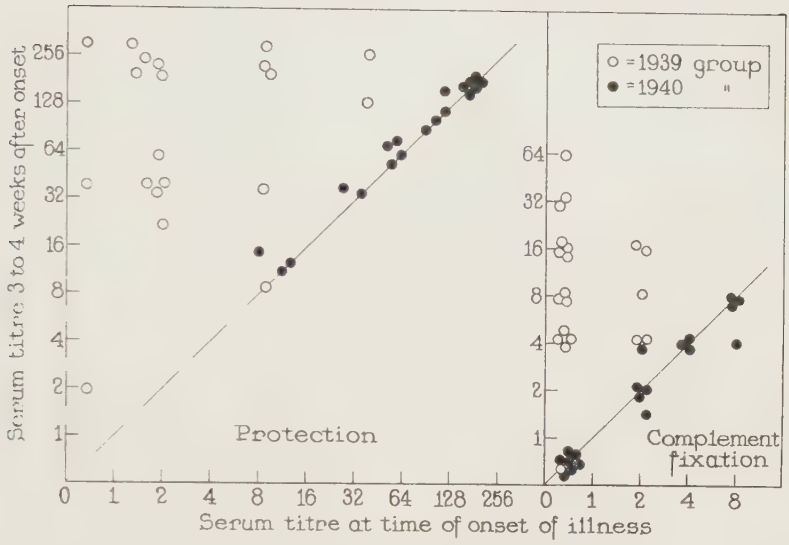

FIG. 1.
Tests with *PR8* virus.

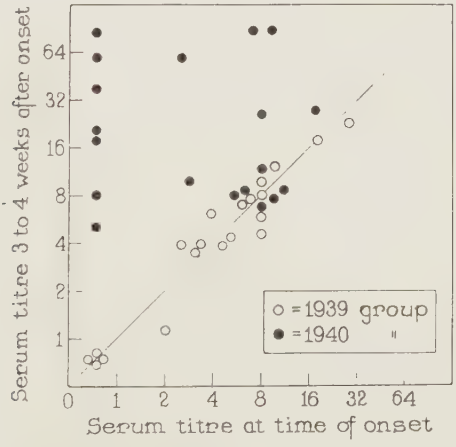

FIG. 2.
Tests with *TM* virus.

3 to 4 weeks later. The titers of the protective antibodies are expressed as the dilution of serum that would protect 50% of the mice from death;[6] all titers represent the actual dilution of serum before the addition of antigen or of antigen plus complement.

In Fig. 1 it is evident that all but one of the 20 subjects of the 1939 outbreak showed a significant increase both in protective and in complement fixing antibodies against the *PR8*, whereas none of

[6] Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.

the 19 subjects of the 1940 outbreak showed any appreciable increase in antibodies against that virus. As shown in Fig. 2, the serums from the subjects of the 2 outbreaks reacted quite differently when tested against the *TM* virus; none of the 1939 group showed any appreciable increase in antibodies, whereas most of the patients of the 1940 group showed a significant rise in titer against that virus. These results indicate that the agent involved in the 1939 outbreak must have been either identical with or closely related to the *PR8*, and the agent involved in the 1940 outbreak either identical with or closely related to the *TM*. Thus, the data furnish an example in which the influenza that occurred in the one year was due to a virus distinctly different in antigenic properties from the virus responsible for the clinically similar disease that occurred in the same institution a year previously.

11997

Excretion of Nicotinic Acid in Pellagra.*†

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The impression is given by preliminary reports from several sources[1, 2, 3] that the excretion of nicotinic acid in pellagra is "not vastly different" from that of healthy individuals, in marked contrast to the excretion of certain other vitamins in their corresponding states of deficiency. This impression is fully confirmed by the results of the present report.

Observations have been made on several patients with deficiency diseases. Among these were cases of classical pellagra; others were states which were probably due to deficiency of several factors. Ob-

* This study was conducted in collaboration with the various members of the staff of the Department of Internal Medicine. Acknowledgment should be made particularly to Dr. J. W. Weaver who administered test doses of nicotinic acid to patients.

† Acknowledgment is also made of aid from the John and Mary Markle Foundation.

1 Kühnau, W. W., *Klin. Woch.*, 1939, **18**, 1333.

2 Swaminathan, M., *Indian J. Med. Res.*, 1939, **27**, 417. (*Nut. Ab. Rev.*, 1940 **9**, 1037.)

3 Harris, L. J., and Raymond, W. D., *Biochem. J.*, 1939, **33**, 2037.

servations are also reported on a group of controls including normal individuals and patients without signs of any deficiency disease, and also 3 patients who were known to have had pellagra in the past.

Methods. Three types of nicotinic acid tolerance test have been employed: (1) The excretion of nicotinic acid in the urine after administration of a small (25 mg) dose by mouth. This was administered at midnight and urine collected from midnight to 6:00 a.m. The test was repeated on 2 successive nights and comparison made of nicotinic acid excretion over similar periods before and after; (2) The urinary excretion for 6 hours after administration of a large oral dose (200 mg nicotinic acid); (3) The urinary excretion for 3 hours after intravenous administration of 25 mg of nicotinic acid.

The avitaminotic patients were maintained on a pellagra producing diet. The three control cases who were known to have had pellagra in the past also received this diet. The other control subjects were unrestricted.

The determination of nicotinic acid was based on the color developed with cyanogen bromide and aniline, after acid hydrolysis, neutralization and clearing with zinc hydroxide. Throughout most of the study hydrolysis was extended for 2½ hours at 95°C with an acidity of 1½ N HCl. Following the appearance of the study of Melnick, Robinson, and Field[4] it was observed that the same results were obtained by hydrolysis for 30 minutes in boiling water with an acidity of 2½ N HCl. Thereafter the shorter procedure was employed, and the results as was pointed out may be taken to include nicotinic acid plus nicotinamid plus two-thirds of any nicotinuric acid which may be present. Zinc hydroxide was employed for removal of pigment at the suggestion of Dr. H. A. Waisman. It was found not to remove a significant quantity of nicotinic acid, and recovery experiments gave results consistently better than 90%. Nicotine was also found to escape removal by this precipitation and to possess about 70% of the color value of nicotinic acid.

Technic of color development was that of Pearson;[5] results were reproducible providing the interval between addition of cyanogen bromide and aniline was timed accurately to 2 minutes. The Evelyn photoelectric colorimeter with the 440 filter has been employed for measurement of color intensity. Since all of the urinary pigment was not removed by treatment with zinc hydroxide, a reading was taken immediately before development of color; the value of this

[4] Melnick, Daniel, Robinson, W. D., and Field, Henry, Jr., *J. Biol. Chem.*, 1940, **136**, 131.

[5] Pearson, P. B., *J. Biol. Chem.*, 1939, **129**, 491.

reading was diminished by the factor of dilution due to addition of aniline and cyanogen bromide and then subtracted from the value of the final reading.

Sodium citrate was added as a buffer, since the color is influenced by changes in acidity. The acidity of the final mixture with this technic is a little less than that of Pearson, and the rapidity of color development a little greater.

The nicotinic acid results in Exp. I were corrected for creatinine excretion; they were multiplied by the factor: average creatinine

TABLE I.

Tests of Nicotinic Acid Tolerance, Employing a 25 mg Oral Dose. Urinary Excretion mg Nicotinic Acid for 6-hr Period.

	Period							
	Fore		Test		After			
Control group	1st day	2nd day	1st day	2nd day	1st day	2nd day		
L.G.	.40	.36	.48	.37	.33	.28	Bronchial Asthma	
G.G.	.23	.20	.39	.29	.16	.26	Rheumatic Heart Disease	
J.R.	1.32	1.65	2.68	2.00	—	—	Arthritis	
R.P.C.	.17	.24	.15	.83	.19	.31	Hypertension	
W.E.	.29	.38	.46	1.51	.19	.29	Bacterial Endocarditis	
C.S.	.57	.35	.29	.59	.57	—	Rheumatic Fever	
I.G.	.22	.31	.33	.52	.29	.24	Arthritis—Healed Pellagra	
W.J.	.73	1.06	1.27	1.15	1.09	1.02	Nephritis "	"
K.G.	.41	.40	.33	.60	.28	.36	Hypertension "	"
Dr.W.	1.29	1.34	1.55	1.46	1.72	—	Normal Control	
Dr.S.	2.36	2.00	2.09	1.70	1.76	—	" "	
Dr.B.	.32	.25	.32	.27	.21	—	" "	
Max.	2.36	2.00	2.68	2.00	1.76	1.02		
Min.	.22	.20	.15	.27	.16	.26		
Mean	.69	.71	.86	.94	.62			
Deficient group								
Cl.	2.19	2.18	4.36	4.02	—	—	Pellagra—Active	
Gl.	2.24	2.41	3.58	2.93	—	—	" "	
M.W.	.06	.12	1.01	1.00	.31	.29	" "	
Wi.	2.78	2.44	2.56	3.06	2.24	1.81	" "	
Bl.	2.89	2.24	2.48	2.07	2.21	2.88	" "	
K.H.	.21	.35	1.30	1.73	.38	.42	" "	
Go.	1.01	1.11	2.03	1.77	—	—	" "	
G.D.	2.67	2.73	2.98	2.14	—	2.75	" ?	
W.S.	.54	.43	1.53	.94	.44	.45	" ?	
P.N.	1.17	.68	.97	1.04	.77	.84	" ?	Ariboflavinosis
Ne.	.26	.26	1.65	1.68	.36	—	" ?	"
La.	.89	1.22	1.25	1.37	1.15	1.12	"	Pit. Cachexia ?
Max.	2.89	2.73	4.36	4.02	2.24	2.88		
Min.	.06	.12	1.01	.94	.31	.29		
Mean	1.41	1.35	2.14	1.98	.98	1.32		

÷ (divided by) daily creatinine. Creatinine was determined with the aid of the photoelectric colorimeter and the Jaffe reaction.

Results. Chief interest attaches to the results of Exp. I. (Table I.) The basal 6-hour excretion of the control group in the fore periods is observed to range from 0.20 to 2.36 mg nicotinic acid. The equivalent 24-hr values of about 1 to 10 mg are of the same order as those which have been reported for complete 24-hr urines.[1–4]

The highest values in the control group were obtained from the subjects, Dr. W., Dr. S., and J. R. Of these Drs. W. and S. were known to smoke at night and the slightly higher results were thought to be due to nicotine. The other subjects of the control group, as well as those of the deficiency group, were instructed not to smoke after 6:00 P.M. Possibly, however, some of the results which seem to be out of line were also due to night smoking.

The basal 6-hour excretions of the 3 healed pellagrins, on a deficient diet, are not lower than those of some of the other members of the control group on unrestricted diets.

It was anticipated that a slight increase in excretion during the test period would be shown by the normal individuals and that the patients with deficiency states would show a lesser increase or perhaps none at all. A glance at the table reveals that nothing of the sort was realized; actually the opposite is more nearly true. The mean increase of the test period over the fore period in the deficiency group is about 0.6 mg, compared to an increase of about 0.2 mg for the control group. Also the mean basal 6-hour excretion of the deficiency

TABLE II.
Additional Tests of Nicotinic Acid Tolerance.

Control group	25 mg Intravenous 3 hr Excretion	200 mg Oral 6 hr Excretion	
Hu.	2.51	10.90	Nephritis, acute
Sm.	1.36	7.11	Cardiac
Fi.	1.03	7.88	Hypertension
J.S.		17.70	Cardiac
A.T.		8.87	Pneumonia
I.G.	1.15	12.25	Arthritis
Gr.	3.14	16.10	”
Jo.	1.93	4.92	Nephritis, chr.
Deficient group			
Bl.	4.33	9.30	Pellagra
K.H.	1.06	3.46	”
Wi.	5.25	18.74	”
W.S.	1.02	15.20	”
P.N.	1.46	16.61	? Ariboflavinosis
Ne.	1.19	12.52	? ”

group is a little greater than that of the controls. However, the differences are not great and probably should not be considered significant, especially since other reports have generally observed slightly lower values for nicotinic acid excretion in the deficient state.[1, 2, 3]

The results of the other 2 tolerance tests are shown in Table II. Again the response of the deficient group is seen to be not significantly different from that of the control group.

It is of some interest to contrast the responses of 2 members of the deficient group (B. and H.). They had a similar pellagrous eruption; they were on the same deficient diet; and each smoked occasionally during the day. H. showed a low basal excretion and a poor response to all tests; B., on each occasion, gave relatively high values.

The similar behavior of the 2 groups must depend on similar levels of plasma nicotinic acid. Melnick, Robinson and Field[6] found a parallel between fluctuations of plasma (free nicotinic acid values) and urinary excretion. They also observed that the plasma level exhibited a tendency to remain constant; only transient fluctuations with nicotinic acid dosage were noted. Since there appears to be no significant difference in regard to the level of nicotinic acid in whole blood between the normal and deficient state,[7, 8] it might be expected to find the basal urinary excretion to be essentially unchanged in pellagra.

Summary. The observations reported here indicate that the urinary excretion of nicotinic acid in pellagra is not significantly different from that of the normal.

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[6] Melnick, Daniel, Robinson, W. D., and Field, Henry, Jr., *J. Biol. Chem.*, 1940, **136**, 157.

[7] Ballif, L., Lwoff, A., Querido, A., Ornstein, I., *Comp. Rend. Soc. Biol.*, 1939, **131**, 903.

[8] Unpublished work in this laboratory.

11998

Relation of Nutrition to Mammary Growth After Estradiol Administration to Hypophysectomized Rats.

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To explain the failure of estrogens to stimulate mammary growth in hypophysectomized animals, as first observed by Lyons and Pencharz,[1] Turner[2] has introduced the hypothesis that the effects of the estrogens and progesterone on the mammary gland are not direct but are due to respectively stimulating the secretion of a duct-growth and an alveolar factor by the anterior lobe of the pituitary gland. On the other hand Lyons, *et al.*,[3] and Speert[4] have shown that if estrogens are administered by inunction over one mammary gland, this gland will show greater development than that on the opposite side. They conclude, therefore, that estrogens must have a direct action on the mammary gland.

The experiments of Astwood, Geschickter and Rausch[5] raised the question of the importance of food intake on mammary development. They restricted intact rats to a food intake comparable to that of hypophysectomized littermates and found the same refractoriness of the mammae to estrogen stimulation.

The hypothesis that the failure of estrogens to act directly on the mammary glands of hypophysectomized animals was due to their state of chronic under-nutrition was given further support by the work of Nathanson, Shaw and Franseen.[6] These workers reported that a growth extract which had no direct effect on the mammary glands enabled estradiol to cause proliferation when given

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1 Lyons, W. R., and Pencharz, R. I., Proc. Soc. Exp. Biol. and Med., 1936, **33**, 589.

2 Lewis, A. A., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bul.* 310, 1939.

3 Lyons, W. R., and Sako, Y., Proc. Soc. Exp. Biol. and Med., 1940, **44**, 398.

4 Speert, H., *Science*, 1940, **92**, 461.

5 Astwood, E. B., Geschickter, C. F., and Rausch, E. O., *Am. J. Anat.*, 1937, **61**, 373.

6 Nathanson, I. T., Shaw, D. T., and Franseen, C. C., Proc. Soc. Exp. Biol. and Med., 1939, **42**, 652.

in conjunction with it. The effect of the estradiol seemed to be roughly proportionate to the gain in weight produced by the pituitary preparation. This experiment, however, did not distinguish between the influence of the extract on nutrition (food intake and absorption) and its influence on the internal metabolism. In the following experiment we have attempted to distinguish these two effects by artificially maintaining the food intake of the hypophysectomized rats at such a level that weight was gained instead of lost. With the nutrition adequately maintained in this manner we have studied the response of the mammary gland to estradiol benzoate.

Three litters of 3 young adult virgin female rats each were selected for the experiment. One rat of each litter was kept as a control and the others were hypophysectomized. All animals were then fed a balanced mixed diet by stomach tube in such quantity that body weight was approximately maintained.[7] Five milligrams of desiccated thyroid was given daily mixed in the diet. The rats received sodium chloride-sodium carbonate solution to drink, to which was added 10 cc of Upjohn's cortical extract per 100 cc solution. Estradiol benzoate, 1000 IU in oil,† was injected every other day for 28 days. Vaginal smears were run daily. All animals showed estrus smears. At the end of this time gross observations showed that the nipples of the control rats were considerably enlarged while those of the hypophysectomized rats were as small as at the beginning. Both hypophysectomized and normal rats had gained weight during the experimental period, the former an average of 19 g (6-38 g) from an average original weight of 242 g and the latter an average of 27 g (21-33 g) from an average original weight of 204 g.

The animals were then killed, skinned, and the skins preserved in Bouin's solution. The individual mammary glands were dissected free with the nipple attached. They were then stained with hemalum and mounted according to the technic of Lewis.[8] All the control rats showed considerable lobular development with wide branching ducts. On the other hand, the glands of the hypophysectomized rats were so small as to be difficult to dissect. They showed a few twigs and branches, but not significant development of either ducts or secretory alveoli. A typical specimen of a gland from each group is shown in Fig. 1.

[7] Reinecke, R. M., Ball, H. A., and Samuels, L. T., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 44.

† The estradiol benzoate was kindly furnished by the Schering Corporation, Bloomfield, N.J.

[8] Lewis, A. A., personal communication.

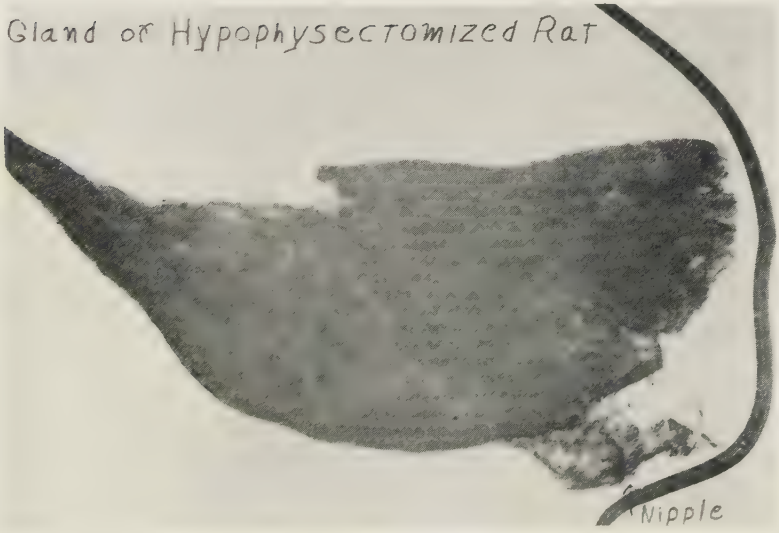

FIG. 1.
Typical Mammary Glands of Hypophysectomized and Control Rats Treated with Estradiol Benzoate.

In both slides the entire fat pad above and including a nipple has been mounted. The pad of the hypophysectomized rat was not destained as much as the control, and the vascular system can therefore be seen. There is obviously no duct system entering the nipple of the hypophysectomized animal.

It seems, therefore, that while the mammary gland may react directly to the stimulation of estradiol, it is essential that there be

present at the same time an active pituitary gland. The supplying of adequate amounts of caloric material is insufficient of itself to enable the mammary gland to respond to estradiol. Either the change in internal metabolism brought about by the growth fraction is essential to mammary development or some specific mammogenic factor is needed.

On the basis of the work of Nathanson, *et al.*,[6] the pituitary factor will apparently only affect the mammae in the presence of estrogens. Since Turner's extracts have not been demonstrated to be estrogen-free, it may be that their effectiveness in hypophysectomized rats was due to the combined presence of small amounts of such substances and some pituitary factor as yet not clearly distinguished. This apparently was present in both Nathanson's and Turner's extracts.

The experiments of Astwood, *et al.*,[5] can best be explained by assuming that the pituitary gland is sensitive to undernutrition. This was first shown by Moore and Samuels[9] in connection with the gonadotropic factor. If this assumption is correct Astwood's rats failed to show mammary development because of lack of the pituitary factor.

Summary. Well nourished hypophysectomized rats did not show mammary development when stimulated by large doses of estradiol benzoate for a period of 28 days. When this result is correlated with those of other workers it appears that both estrogens and some pituitary factor must act directly on the mammary gland to produce normal development.

11999 P

Efficiency of Electrical Energy Production by Surviving Frog Skin, Measured by Iodine Coulometer.*

PAUL STAPP. (Introduced by Ancel Keys.)

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Surviving frog skin is the seat of an electrical potential which has been related to factors affecting its metabolic activity.[1, 2] To deter-

[9] Moore, C. R., and Samuels, L. T., *Am. J. Physiol.*, 1931, **96**, 278.

* Based on data submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Texas, Austin, Texas.

[1] Lund, E. J., *J. Exp. Zool.*, 1926, **44**, 383; 1928, **51**, 291, 327; 1931, **60**, 249.

[2] Francis, W. L., *Nature*, 1933, **131**, 805.

mine the efficiency of electrical energy production through an external circuit by surviving frog skin it is necessary to measure the joules of electrical energy and the oxygen consumption of the skin during the same interval of time. An iodine coulometer[3] of low resistance (18-22 ohms) with sufficient electrode area to minimize polarization within the range of skin potentials, was employed to integrate the quantity of current passing through the external circuit. The coulometer was calibrated against known, constant current densities for measured intervals with a microammeter in series as a check. Average voltage was determined with a standard potentiometer by readings at 5 to 10 minute intervals.

The oxygen consumption was found by difference between initial and final oxygen concentrations in 25 ml samples of the Ringer's solution surrounding the skin, using the Winkler method for analysis.

Experiments were carried out in duplicate on symmetrical pieces of frog skin *(Rana catesbiana)* of 24 cm^2 area. Each piece of skin was clamped between a pair of iso-electric cup electrodes with 60 ml of Ringer's solution on each side. The electrodes were of lead amalgam-lead chloride covered by a surface layer of saline agar. The external circuit consisted of Ringer's solution, the electrodes, a coulometer and a microammeter in series. A potentiometer was connected across the electrode terminals.

Results of a typical one-hour determination at 25°C: The coulometer measured 1.497 coulombs. The average potential for 6 readings was 0.039 volts. The oxygen consumption was 0.239 ml at standard temperature and pressure. The respiratory quotient of the isolated skin was not determined. The highest and lowest respiratory quotients for the intact frog range from 0.94 to 0.72.[4] Calculations of the efficiency based on calorie equivalents for each of these respiratory quotients were found to differ relatively little. From the equation

$$\frac{EIT/4.181 \times 100}{\text{ml. } O_2 \times C} = \%\ \text{Efficiency}$$

Where E = average volts, IT = coulombs, 4.181 = factor converting joules to calories, and C = calorie equivalent for a given respiratory quotient. Substituting experimental values in this equation:

$$\frac{0.039 \times 1.497/4.181 \times 100}{0.239 \times 4.702} = 1.0\% \text{ for a R.Q. of } 0.94.$$

[3] Washburn, E. W., and Bates, S. J., *J. Am. Chem. Soc.*, 1912, **34**, 1341.

[4] Dolk, H. E., and Postma, N., *Z. f. Vergl. Physiol.*, 1927, **5**, 417.

The corresponding value for the R.Q. of 0.72 gave a value of 1.06%. The chemical efficiency was determined from the relation:

$$\frac{\text{Thiosulfate equivalent of coulombs} \times \text{volts} \times 100}{\text{Thiosulfate equivalent of oxygen}} = \text{Efficiency}$$

Substituting experimental values:

$$\frac{1.625 \times 0.039 \times 100}{4.49} = 1.23\%.$$

The control determination gave values of 0.93% for a 0.94 R.Q., 0.99% for a 0.72 R.Q., and 1.13% for the chemical efficiency. Average values for 20 experiments are shown in Table I.

TABLE I.

No. of Exps.	Duration	Avg volts/hr	Coulombs per hr	Gram calories per hr	Cc O_2/hr at standard T. and P.	Efficiency from R.Q. %		Chemical Efficiency
						0.72	0.94	
14	2 hr	.0235	1.134	.0068	.130	1.10	1.04	1.20
3	1 hr	.0260	1.208	.0086	.140	1.37	1.30	1.58
3	30 min	.0346	1.671	.0107	.132	2.50	2.38	2.87

Other determinations made with external circuit resistances varying from 65 to 1500 ohms indicate that electrical energy output and efficiency diminish with rising resistance. If we assume that the external circuit resistance of 65 ohms is approximately equal to the internal resistance of the skins in the 30-minute determinations (Table I), the total electrical energy output of the skin would be in the neighborhood of 5 or 6% of the total energy metabolism. A gradual fall of the energy production curve during the first 30 minutes, followed by a steeper decline during subsequent intervals accounts for the higher efficiency in the shorter determinations. Exposure to saturated air for 8 to 10 hours caused a 40 to 50% increase in energy production during the succeeding 1- to 2-hour determinations. That the decline in energy production was not necessarily related to a continuous external short circuit was shown by a similar decline in a skin whose external circuit was closed only for microammeter readings.

Further investigation of the effect of varying oxygen tension, temperature and previous state of nutrition on the energy output are being undertaken to elucidate the nature of the relation between metabolic processes and electrical energy output of the frog skin.

12000 P

Effect of Slowly Absorbed Epinephrine in Experimental Shock.*

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The impression is prevalent that administration of adrenalin is harmful in clinical shock.[1] Furthermore, various investigators including Freeman[2, 3] have reported that a condition which they claim is analogous to shock can be produced in experimental animals by continuous injection of large doses of adrenalin. However, other workers have been unable to produce shock by prolonged administration of this drug.[4, 5] In a recent study of traumatic shock,[6] physiologic doses of adrenalin were shown to have a more pronounced pressor action in the course of shock than before trauma.

In view of the controversy concerning the rôle of epinephrine in shock, the effect of administration of slowly absorbed epinephrine in experimental shock was investigated. Shock was produced in anesthetized cats by exposure and manipulation of the small intestine for 30 minutes. The manipulation consisted of stripping the gut forcefully throughout its length. In the control experiments this procedure produced a marked momentary spasm of the segment of gut stripped. There was considerable oozing of sanguinous fluid and the bowel became purple in color. The visible fluid loss at the time of manipulation never exceeded 5 cc. In the animals receiving adrenalin, the identical procedure resulted in less spasm, no visible fluid loss, and less discoloration of the gut.

In 14 cats, anesthetized with chloralose, 80 mg/kilo, the bowel was manipulated without aseptic precautions, using bare hands, and blood pressure was recorded with a mercury manometer from a cannula in the carotid artery. In 6 control experiments, a fall in

* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

1 Moon, V. H., *Shock and Related Capillary Phenomena*, Oxford University Press, 1938, p. 391.

2 Freeman, N. E., *Am. J. Physiol.*, 1933, **103**, 185.

3 Freeman, N. E., Freedman, H., and Miller, C. C., *Am. J. Physiol.*, 1941, **131**, 545.

4 Hamlin, E., and Gregerson, M. I., *Am. J. Physiol.*, 1939, **125**, 713.

5 Van Prohaska, J., Harms, H. P., and Dragstedt, L. R., *Ann. Surg.*, 1937, **106**, 857.

6 Freedman, A. M., and Kabat, H., *Am. J. Physiol.*, 1940, **130**, 620.

blood pressure to shock level (60 mm Hg) was produced in an average of one hour and 33 minutes after beginning intestinal trauma, with a range from 35 to 195 minutes. These animals survived an average of 3 hours with a range from 47 minutes to 4 hours and 23 minutes.

Eight experiments similar to the above were performed using slowly absorbed epinephrine. The preparations used were adrenalin in peanut oil and suprarenalin-gelatin for which we are indebted to Parke, Davis & Co. and Armour and Co., respectively. The drug was administered intramuscularly in doses of 2 mg per kilo 2-3 hours preceding manipulation. Following intestinal trauma, 2 mg of epinephrine was injected every 2-3 hours until the original dose had been repeated (6-10 hours). The blood pressure at the time manipulation was begun averaged 154 mm Hg, with a range from 126-167 mm Hg. Thus "slow" adrenalin caused only a slight rise in blood pressure which was well maintained. In 6 of the 8 experiments, blood pressure was maintained at a normal level during and following manipulation of the bowel for many hours. In these experiments blood pressure fell to shock level in an average time of 8 hours and 7 minutes, with a range from 6 hours and 10 minutes to 10 hours. In 2 unsuccessful experiments with "slow" adrenalin, shock supervened in 1½ hours.

In another series of 36 experiments, intestinal manipulation was carried out aseptically, the operator wearing rubber gloves. Blood pressure was not recorded. These cats were anesthetized with nembutal, the usual dose being 25 mg/kilo intravenously. In 14 controls the average survival was 12.9 hours and the median survival was 9 hours. Except for one instance in which the cat survived for 65 hours, the survival ranged from 1 hour and 17 minutes to 17 hours and 24 minutes. At autopsy, no gross pathology was found except in the manipulated gut.

In 22 cats to which "slow" epinephrine was administered as described previously, aseptic manipulation was carried out with gloved hands. The average survival in these animals was 42.7 hours and the median survival was 27 hours. The range of survival was from 16 hours and 30 minutes to 192 hours (8 days). The longest survivals were for 8 days, 6 days, 4 days, and 2½ days. All of the "slow" epinephrine series survived longer than the average or the median of the controls. Postmortem examination revealed the cause of death in 7 animals was pneumonia, in 2 peritonitis, and in the remaining 13 cats the pathology was confined to the manipulated gut.

These experiments indicate, therefore, that "slow" epinephrine will maintain the blood pressure during and following intestinal manipulation and will increase survival 300%. This confirms the encouraging clinical results reported in shock using vasoconstrictor drugs such as adrenalin,[7] ephedrine,[8] and neosynephrine.[9] Recent experiments of Best and Solandt[10] indicate that adrenalin and other vasoconstrictor drugs may be a valuable adjunct in the therapy of some forms of shock.

12001

Mechanism of Intestinal Absorption of Thiamin.

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We have reported[1] that the intestinal absorption of substances whose absorption is accelerated by phosphorylation is specifically stimulated by thyroxin. Banga Ochoa and Peters[2] have demonstrated that the phosphorylated form of thiamin, cocarboxylase, is active in pyruvic acid oxidation. Therefore it seemed of interest to ascertain whether thyroxin would accelerate the absorption of thiamin from the digestive tract.

Apparently a close relation exists between thyroxin and vitamin B because the requirement for vitamin B increases when metabolism is augmented. Cowgill[3] and his coworkers have shown that the need for the vitamin B complex is greatly increased when metabolism is accelerated experimentally by the administration of thyroxin. Drill[4] has demonstrated that thiamin injections offset the loss of weight in rats caused by feeding of thyroid substance. Furthermore, Peters and Rossiter[5] have found that hyperthyroidism causes a fall in tissue

7 Mummery, J. P. Lockhart, *Lancet*, 1905, **1**, 846.

8 Johnson, C. A., *J. A. M. A.*, 1930, **94**, 1388.

9 Johnson, C. A., *Surg. Gyn. and Obst.*, 1937, **65**, 458.

10 Best, C. H., and Solandt, D. Y., *Brit. Med. J.*, 1940, **1**, 799.

1 Althausen, T. L., and Stockholm, M., *Am. J. Physiol.*, 1938, **123**, 577.

2 Banga, I., Ochoa, S., and Peters, R. A., *Biochem. J.*, 1939, **33**, 1109.

3 Himwich, H. E., Goldfarb, W., and Cowgill, G. R., *Am. J. Physiol.*, 1932, **99**, 689.

4 Drill, V. A., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 313.

5 Peters, R. A., and Rossiter, R. J., *Biochem. J.*, 1939, **33**, 1140.

cocarboxylase and thiamin. Clinically, Parade[6] has shown that feeding of thyroid substance aids in alleviating the symptoms of vitamin B_1 deficiency and Frazier and Ravdin[7] have shortened the preoperative preparation of hyperthyroid patients by treating them with thiamin.

Experimental Procedure. Female rats weighing between 200 and 250 g were employed in these experiments. A group of these animals was rendered hyperthyroid by daily intraperitoneal injections of 0.1 mg thyroxin per 100 g of body weight for 12 days. The average increase of the basal metabolic rate of rats so treated, as determined in a closed circuit apparatus similar to that used by Benedict and Macleod,[8] was nearly 50%. All animals were fasted for 48 hours in order to clear their intestinal tracts of food residues. Since rats in the hyperthyroid state are adversely affected by prolonged fasts, each hyperthyroid animal was given a cube of sugar after 24 hours of fasting. Both normal and hyperthyroid animals were then given 100 μg thiamin chloride in 2 cc of water by stomach tube. Normally rats require only 10 μg of thiamin daily. At various intervals the animals were sacrificed and the amount of unabsorbed residue of thiamin in the digestive tracts was determined. In order to observe the effects of a still larger dose, 1 mg of thiamin was administered to normal and hyperthyroid rats in the manner described.

The thiamin was estimated by a simplification of the thiochrome method previously described by Borson.[9] Since the quantity of interfering substances is negligible, it was possible to proceed to the oxidation without preliminary adsorption onto Lloyd's reagent. From 0.2 to 1.0 ml aliquots were used, depending on the amount of thiamin present. Protection against destruction of thiamin in the residues was insured by bringing the solution to a pH of about 4.5 and adding toluol. Material preserved in this way retains thiamin indefinitely. The total volume of the washings was 200 ml. The original body weight before administration of thyroxin was chosen as a basis for calculation of the rate of absorption.

Results. The figures showing absorption of thiamin in normal and in hyperthyroid rats after administration of 100 μg are given in Table I. From a study of these figures it appears that in normal fasting rats an initial period of rapid absorption (56 μg per hour)

[6] Parade, G. W., *Z. Vitaminforsch.*, 1938, **7**, 35.

[7] Frazier, W. D., and Ravdin, I. S., *Surgery*, 1938, **4**, 680.

[8] Benedict, F. A., and Macleod, F., *J. Nutr.*, 1929, **1**, 343.

[9] Borson, H. J., *Ann. Int. Med.*, 1940, **14**, 1.

TABLE I.
Absorption of Thiamin Chloride in Normal and in Hyperthyroid Rats after Administration of 100 μg per 100 g of Weight.*

Absorption time	Normal rats		Hyperthyroid rats	
	Amt absorbed, μg	Rate absorption per hr, μg	Amt absorbed, μg	Rate absorption per hr, μg
15 min	14 ± 4†	56.0	18 ± 2†	72.0
30 "	15 ± 1	4.0		
1 hr	18 ± 3	5.3		
2 "	21 ± 2	4.0	24 ± 2	3.5
3 "	28 ± 2	5.1		
4 "	30 ± 4	4.3		

*Each figure represents the average of 6 rats.
†Standard deviation.

is followed by a considerably diminished and almost constant rate of absorption (about 5 μg per hour). The hyperthyroid rats did not absorb a significantly larger amount of thiamin than did the normal animals. When 1 mg of thiamin was administered, the average absorption for a 1-hour period was 108 ± 18 μg per 100 g of body weight in 4 normal rats and 116 ± 25 μg in 4 hyperthyroid rats.

The method described estimates thiamin only. Preliminary hydrolysis with taka-diastase did not increase its recovery in either the normal or the hyperthyroid rats. It was concluded, therefore, that thiamin is not phosphorylated in the lumen of the gut before absorption.

Discussion and Conclusions. Our data show that injections of thyroxin do not increase the rate of intestinal absorption of thiamin chloride in rats as they do in the case of dextrose and other substances susceptible to phosphorylation. This observation would indicate that phosphorylation plays no dominant part in determining the rate of intestinal absorption of thiamin or that thyroxin has no stimulating effect on the rate of enzymic formation of the pyrophosphate ester linkage. The stimulating effect of thyroxin may be limited to the phosphorylating processes involving the mono-phosphoric acid ester, as in the transport of dextrose across the intestinal mucosa. The conclusion that phosphorylation plays no part in the intestinal absorption of thiamin is supported also by the work of Ochoa,[10] who found that *in vitro* intestinal tissue, in contrast to liver tissue, possesses little ability to phosphorylate thiamin.

Two observations made during the course of our experiments indicate that absorption of thiamin in the intestine probably takes place by means of simple diffusion. First, the rapid initial absorption

10 Ochoa, S., *Biochem. J.*, 1939, **33**, 1262.

of thiamin is succeeded by a much slower rate of absorption, presumably after the diffusion equilibrium between the lumen of the gut and the intestinal mucosa has become established. Secondly, absorption of thiamin is roughly proportional to its concentration in the intestine, as was seen when the dose of thiamin was increased from 100 μg to 1 mg. On the other hand, if the absorption of a substance is aided by phosphorylation, as, for instance, is the case with dextrose, the rate of absorption remains constant and is within wide limits independent of the concentration of the substance which is being absorbed.

12002

Relationship Between Sugar Concentration and Glycogenetic Action of Insulin on Rat Diaphragm *in vitro*.

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Previous work has shown that the rate of glucose utilization by the peripheral tissues of both normal and depancreatized dogs depends on the blood sugar level.[1, 2] Insulin appears to act as a catalyst in this process, accelerating a reaction which proceeds at a slower rate in its absence. However, if the blood sugar be sufficiently raised the absence of insulin makes little difference.

The present work is a study of the relationship between the action of insulin and glucose concentration in respect to the storage of muscle glycogen. Using the technic of Gemmill[3] we have observed the relationship of sugar concentration to glycogen formation in rat diaphragm *in vitro*, with and without added insulin.

Methods. Normal fed rats weighing between 80 and 100 g were used for all experiments. The animals were killed by a blow on the head, the diaphragms were quickly removed, trimmed, divided into approximately equal halves, weighed while moist, and introduced into Warburg vessels. The medium used was Hastings'[4] Ringer

* Aided by the Max Pam Fund for Metabolic Research.

1 Soskin, S., and Levine, R., *Am. J. Physiol.*, 1937, **120**, 761.

2 Dye, J. A., and Chidsey, J. L., *Am. J. Physiol.*, 1939, **127**, 745.

3 Gemmill, C. H., *Bull. Johns Hopkins Hosp.*, 1940, **66**, 232.

4 Hastings, A. B., Muus, J., and Bessey, O., *J. Biol. Chem.*, 1939, **129**, 295.

buffered with phosphate or bicarbonate at pH 7.4. The gas phase was 100% O_2, or 95% O_2, 5% CO_2, depending upon the buffer. The insulin employed was an amorphous preparation low in zinc, containing 22 units per mg.* One to 3 units were added per vessel. The total volume in all cases was 3 ml. The incubation period was 3 hours at 38°C. At the conclusion of the experimental period, KOH was pipetted into the main chamber to stop the reaction. The contents of the vessels were quantitatively removed and glycogen was determined by the method of Good, Kramer, and Somogyi.[5]

Inasmuch as Gemmill had already shown that the insulin effect on glycogen deposition was specific, we omitted controls with inactivated insulin preparations or non-specific proteins.

Results. The results are detailed in Table I and graphically illustrated in Fig. 1. The 2 values compared in the horizontal lines

TABLE I.
Glycogen Deposition in Rat Diaphragm *in vitro*, at Different Glucose Concentrations, with and without Insulin.

Rat No.	Initial	100 mg % glucose	Rat No.	100 mg % glucose	100 mg % glucose + insulin
1	133	147	6	285	490
2	90	60	7	110	350
3	210	100	8	130	300
4	130	110	9	60	145
5	127	125	10	100	460
			11	125	285
			12	137	591
			13	158	450
Avg	138	90		138	384
Avg % increase due to sugar				0	
Avg % increase due to insulin					178

Rat No.	200 mg % glucose	200 mg % glucose + insulin	Rat No.	400 mg % glucose	400 mg % glucose + insulin
14	219	282	20	480	450
15	257	461	21	370	540
16	123	366	22	520	320
17	253	294	23	132	160
18	90	400	24	271	323
19	100	150	25	200	450
			26	350	220
			27	570	720
Avg	174	326		362	398
Avg % increase due to sugar	26			162	
Avg % increase due to insulin		110			26

* We are indebted to the Eli Lilly Company for supplies of this insulin.

5 Good, C. A., Kramer, H., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 485.

of each section of Table I, represent the 2 halves of the diaphragm of a single rat. It will be seen that no glycogen was deposited at a glucose concentration of 100 mg % in the absence of added insulin. As the glucose concentration was raised glycogen was deposited in increasing amounts. At a glucose concentration of 400 mg % the final glycogen content reached 3 times the initial value.

The catalytic activity of insulin was very great at a glucose concentration of 100 mg %, still significant at 200 mg %, and negligible at 400 mg %.

It may also be of interest to record that our results resembled Gemmill's, in that insulin did not increase the O_2 consumption of the diaphragms. The average QO_2 with insulin was 7% less than the control.

Discussion. Our results show that in the absence of added insulin the rate of glycogen formation in the diaphragmatic muscle of rats *in vitro* depends upon the concentration of sugar in the medium. Cori and Cori[6] have shown a similar relationship between liver glycogen deposition and blood sugar concentration *in vivo*. Our results further indicate that, in the presence of added insulin, glycogen formation in the diaphragm *in vitro* occurs at a high rate even at comparatively low sugar levels. As the sugar concentration is increased the catalytic effect of insulin becomes less and less evident.

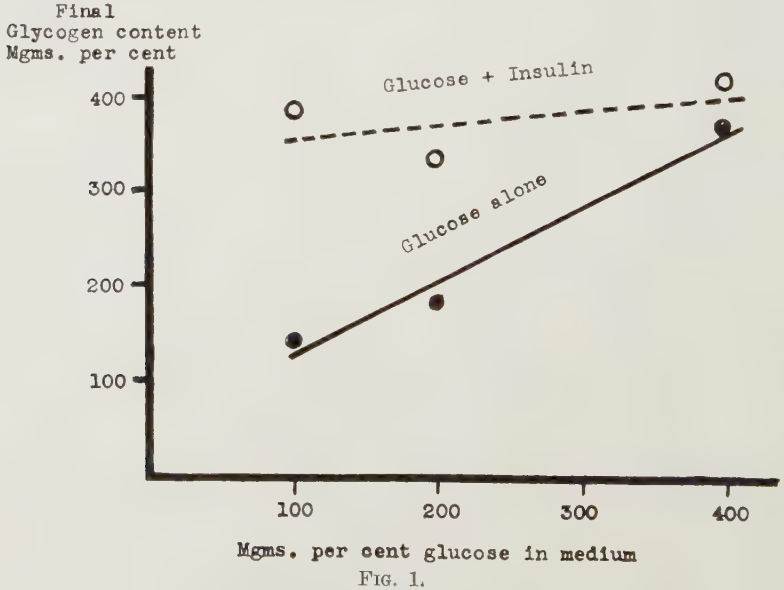

FIG. 1.

[6] Cori, C. F., and Cori, G. T., *J. Biol. Chem.*, 1929, **85**, 275.

The dispensability of insulin at high sugar concentrations is in agreement with the well established evidence that completely diabetic animals can store some glycogen if high blood sugar levels are maintained.[7, 8]

The relationship between the action of insulin and sugar concentration demonstrated for glycogen deposition in the present work, is similar to the relationship previously demonstrated for glucose utilization *in vivo*.[1] The presence of this same relationship in both instances is in agreement with our conception that insulin acts by catalyzing the conversion of glucose into some intermediary metabolite which precedes and is necessary for both glycogen formation and sugar utilization.[9] Thus insulin influences the rate at which sugar enters into the metabolic processes of the cell, but the fate of the sugar which has entered depends on other factors, and insulin cannot be regarded as being a *specific* accelerator of either the storage or the oxidation of carbohydrate.

Summary. Glycogen deposition in rat diaphragm *in vitro* varies directly with the concentration of glucose in the medium. Insulin catalyzes this process greatly at low sugar concentrations but very little at high concentrations. The bearing of these results on the mechanism of insulin action is briefly discussed.

12003 P

Effect of Shearing Forces on the Cortex of the Fertilized Egg of *Arbacia punctulata.*

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In previous communications[1, 2] the theory was advanced that, after monaster formation, the cortex of the fertilized egg of *Arbacia punctulata* consists in part of a bound calcium compound. Data were presented showing that this labile combination can be broken down in the living cell by various chemical agents, with the subsequent release

7 Major, S. B., and Mann, F. C., *Am. J. Physiol.*, 1932, **102**, 409.

8 Barker, S. B., and Sweet, J. E., *Science*, N. S., 1937, **86**, 270.

9 Soskin, S., and Levine, R., *Am. J. Physiol.*, 1940, **129**, 782.

1 Churney, L., *Anat. Rec. Suppl.*, 1938, **72**, 65.

2 Churney, L., and Moser, F., *Physiol. Zool.*, 1940, **13**, 212.

of free calcium ions. For detecting calcium release, use was made of the observation that the characteristic red pigment "granules" of the egg disintegrate in the presence of calcium or other divalent cations.[3] The purpose of this paper is to describe briefly two observations which indicate that, in addition to the chemical procedures discussed in the previous papers, simple physical means are capable of breaking down the cell cortex and causing calcium release.

When immature eggs, or mature unfertilized eggs, or fertilized eggs that have not reached the monaster stage of development, are slowly crushed by withdrawing the sea water from under the cover slip with filter paper, considerable flattening of the egg can occur without rupture of the cell membrane. The compression can attain such a degree that the cell in many places is only a few micra thick. The red pigment granules remain intact as long as the cell membrane is unbroken. The moment that the cell membrane ruptures, the sea water and protoplasm intermingle and the red chromatophores disintegrate. The results are quite different when fertilized eggs that have reached the monaster stage (10-12 minutes after fertilization) are compressed in a similar manner. The slightest pressure on this egg causes the red pigment granules to "explode" in the region of the cell where the pressure is applied. The cell membrane remains intact and many of the red chromatophores are unbroken. The position of each granule that has disintegrated is marked by the appearance of a clear vacuole. This observation is important as indicating that a reaction has taken place between the granule and a divalent cation. With the application of additional pressure more and more granules disintegrate, until the cell is entirely devoid of them. Yet the degree of flattening of such a cell may be very much less than that of a crushed unfertilized egg in which all the red chromatophores are still intact.

The same general results hold for eggs centrifuged for a short time at low speed; only cells that have reached the monaster stage of development will release pigment to the supernatant fluid.*

In attempting to explain these phenomena the following facts are pertinent. Pigment release is caused by the application of extremely mild centrifugal or shearing forces only after the egg has reached

[3] Heilbrunn, L. V., *Biol. Bull.*, 1934, **66**, 264.

* At certain times of the season the cells are very fragile and centrifuging causes release of pigment from unfertilized eggs. That this is an entirely different phenomenon from the one under consideration is seen in the fact that the supernatant fluid is pink rather than orange. The pink color is typical of cytolyzed eggs in the presence of a high concentration of calcium ions. Such eggs were never used in the experiments.

a certain period of development. At no previous time are these procedures effective; not even when the magnitude of the forces is very much greater. This period of susceptibility coincides with the time of localization of the red pigment granules in a differentiated cortical layer of the cell. Hence it may be deduced that these forces do not act directly on the granules themselves, and it is concluded that the shearing forces act upon the "ground substance" (cortex) in which the granules are imbedded. In doing so they cause the bound calcium component of this cortex to disintegrate. The release of calcium ions is then reflected in the breakdown of the red pigment granules.

12004

Influence of Temperature on the Electrogram and Monophasic Action Potential of the Mammalian Heart.*

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Earlier studies in this laboratory demonstrated that warming or cooling portions of the external surface of the ventricles caused characteristic alterations in the T wave of the electrocardiogram.[1] The basis of these T wave changes was found to be alteration in the duration of one or the other of the two components of the electrocardiogram, *i. e.*, of the dextro- or the levocardiogram. Cold prolonged and heat shortened the duration of these components. The following experiments were undertaken to determine the cause of these alterations in the duration of the dextro- and levocardiograms.

Electrograms and monophasic action potentials were studied in 5 dogs by the method previously described,[2] one lead being taken from the anterior surface of the right ventricle and the other from the anterior surface of the left ventricle. Negativity at the lead on

* This work was aided by a grant from the Fluid Research Funds of the Yale University School of Medicine, and by a Fellowship from the Emanuel Libman Fellowship Fund.

† Fellow in Physiology, Dazian Foundation.

1 Hoff, H. E., and Nahum, L. H., *Am. J. Physiol.*, 1941, **131**, 700.

2 Hoff, H. E., and Nahum, L. H., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 263.

the right ventricle gave an upward deflection of the beam. The influence of temperature was determined first on the electrogram, and then on the monophasic action potential. Variations in temperature were produced by applying a small tin chamber to the surface of the heart under one or the other electrode, and circulating through the chamber water at 5°C or 55°C.

The results of heating or cooling the surface of the heart under one or the other electrode are shown in Fig. 1. When the region under the right ventricle electrode was cooled, an upright end deflection was obtained, while when this region was warmed, the end-deflection was sharply inverted. The duration of the whole electrogram was prolonged when the heart was cooled, and was not materially altered by warming. Exactly opposite effects followed cooling and heating under the electrode on the left ventricle. Cooling produced a prolonged downward end-deflection, while heating produced an upward end-deflection of normal duration.

Monophasic action potentials were obtained from each of the two regions from which the electrogram was recorded by successively

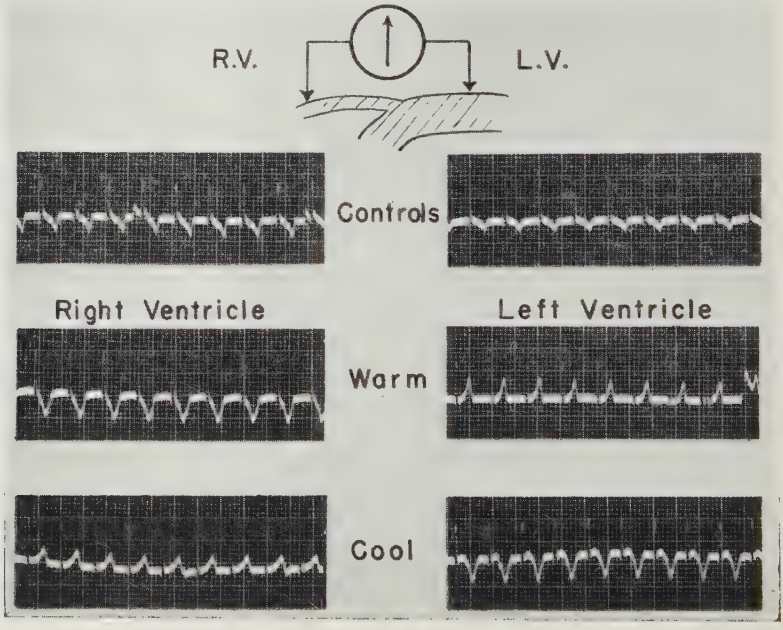

FIG. 1.
Electrograms recorded from the right ventricle (region of primary negativity) and the left ventricle, which was activated later. The effect of warming and cooling the region of one and the other electrode by means of a thermal chamber at 55°C and 5°C compares closely with the hypothetical diagram in Fig. 3.

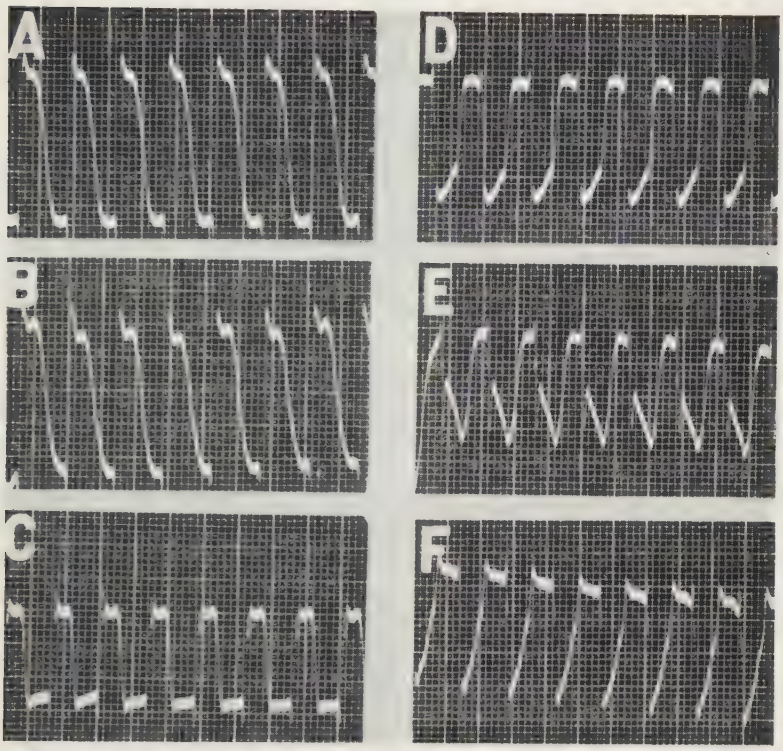

Fig. 2.

Monophasic action potentials recorded from the right ventricle (A, B, C), and the left ventricle (D, E, F), as in Fig. 1, but in another experiment. Cooling and warming the region from which the monophasic action potentials are derived lengthens (B, E) and shortens (C, F) the duration of the waves in comparison with the normal (A, D). This figure, and Fig. 1, demonstrate the correctness of the hypothesis presented in Fig. 3.

blocking one and then the other region with isotonic KCl, as described previously.[2] Fig. 2 shows the results of heating and cooling the regions from which the monophasic records were obtained. It can be seen that cooling (B, E) prolonged the action potential, while warming (C, F) curtailed it.

Fig. 3 illustrates the production of the electrogram by the interference (*i. e.,* algebraic summation) between the monophasic action potentials at the two electrodes, and shows how alterations in one of these two components must alter the end-deflection of the electrogram. In A both complexes (A1, A3) are of equal duration, and therefore the region under the electrode first activated has completely recovered while the tissue under the second electrode is still active, giving an inverted end-deflection (A2). In B recovery under the

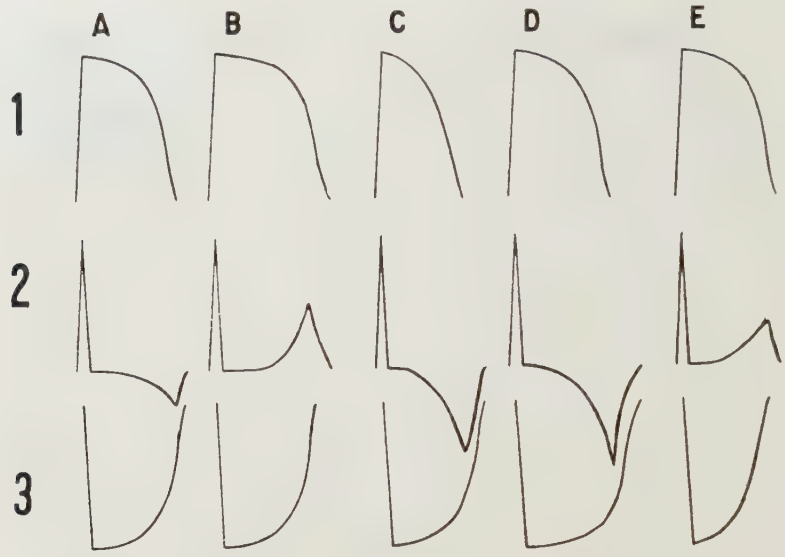

FIG. 3.

Graphic illustrations of the interference theory as applied to the genesis of the electrogram. Above (1) are shown monophasic action potentials as they might be recorded at the electrode first activated, and below (3) as they would be recorded at the second electrode. They would be opposite in polarity as shown, and the action potential at the second electrode would start somewhat later than at the first electrode. If the waves were of equal duration, then the end-deflection of the electrogram which results from the summation of the two would be negative (A). In B is shown the effect of cooling the heart at the first electrode. The monophasic electrogram at this region would be prolonged, and an upward end-deflection would result. In C the region of the first electrode is warmed, shortening the action potential, and accentuating the negativity of the end-deflection. In D and E are shown the results of cooling and heating the region of the second electrode, while the region of the first electrode remains unaltered.

first electrode (B1) is prolonged by cold, so activity persists here after termination of recovery at the second electrode (B3). This produces an upright end-deflection with prolongation of the whole complex (B2). In C, is illustrated the type of end-deflection produced by warming the region under the first electrode, while in D and E are portrayed the effects of cooling and warming the heart at the second electrode.

These experiments, and their interpretation, follow closely those of Burdon-Sanderson and Page,[3, 4, 5] who studied electrograms of the frog and tortoise heart, and who first suggested that differences in the rate of recovery at one or the other electrode will produce changes in the end-deflection.

[3] Burdon-Sanderson, J., and Page, F. J. M., *J. Physiol.*, 1879-80, **2**, 384.

[4] Burdon-Sanderson, J., and Page, F. J. M., *Proc. Roy. Soc., Lond.*, 1878, **27**, 410.

[5] Burdon-Sanderson, J., and Page, F. J. M., *J. Physiol.*, 1883, **4**, 327.

These results provide an explanation for the changes in the dextro- and levocardiograms produced by temperature. It is demonstrated that alterations in the rate of recovery at an isolated region of the surface of the heart result from changes in temperature. When a large part of a single ventricle is similarly heated or cooled, the rate of recovery would be changed over the entire region, and this would be reflected in an alteration in the record of electrical activity from this ventricle, *i. e.*, by a change in duration of the dextro- or levocardiogram.

It should be pointed out that the monophasic records as derived in experiments of this type contain some conducted effects from portions of the ventricles at a distance from the electrodes. In the experiments reported here these conducted effects were unaffected by heating and cooling, since this treatment was restricted to the immediate area of the electrode. The changes recorded in the monophasic action potentials could, therefore, have been produced only by alterations of the action potential derived from the immediate vicinity of the active electrode.

12005

Effect of Pro-Oxidants upon Reproduction in Rats.

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Vitamin E is readily destroyed by oxidation in the presence of autoxidizing fats and this destruction can be prevented by suitable antioxidants or stabilizers. This oxidation is responsible for the production of muscle dystrophy in herbivora on certain diets containing cod liver oil. To insure the complete absence of vitamin E from diets designed to produce a deficiency in rats, the mixed rations are often allowed to stand at room temperature some days before use. Whether pro-oxidants can also accomplish the destruction of vitamin E in the tissues, or otherwise interfere with the normal progress of reproduction, is uncertain.

Waddell and Steenbock[1] believed that the "antivitamins" produced in a diet treated with ethereal ferric chloride damaged the reproductive capacity of female rats, because 4 to 6 weeks elapsed

1 Waddell, J., and Steenbock, H., *J. Nutrition*, 1931, **4**, 79.

(on normal food) before the animals recovered from the effects of this diet. More recent observations,[2, 3] however, indicate that even highly pro-oxygenic substances can be fed without immediate derangement of reproduction, provided the vitamin and pro-oxidant do not meet in the alimentary tract.

According to Kudrjashov and his coworkers,[4] variously prepared decomposition products, in the unsaponifiable fraction of fats undergoing rancidity, when fed to pregnant rats (and rabbits) on normal diets tended to liquidate the pregnancy by causing the death of implanted embryos or by interfering with implantation. Subcutaneously administered concentrates were effective in one-tenth the amount given by mouth and the feeding of large amounts of rancid fats also interrupted pregnancy. Rats were most susceptible immediately after implantation, on the 6th to 9th days, and the placental sign appeared at the 8th to 10th day instead of at the normal time. Ovaries and uterus were undamaged. From numerous observations, some of which are contradictory and not too well controlled, Kudrjashov concluded that vitamin E and the decomposition products of fat are not antagonistic *in vivo,* that these products, presumably higher aldehydes and ketones, do not destroy the vitamin E of the tissues but have a direct toxic action on the fetus.

More recently[5] he produced the same results by feeding or injecting ethereal-ferric chloride treated wheat germ oil. If, after a resorption so produced, females were immediately transferred to a vitamin E deficient diet and mated, litters were born normally, indicating again that tissue vitamin E had not been destroyed, and that embryonic death was due to direct toxic action of the products of rancidity.

The work whose contrary results are reported here, had been under way during 2 years, as opportunity offered, when Carruthers[6] described the effects of heptaldehyde on mice. The daily feeding of 40-50 mg of heptaldehyde to mice, beginning from the first to the fourth day after insemination and continuing until birth or resorption, was followed by a resorption in more than half the animals. Resorptions could also be produced by daily intraperitoneal injections of 0.02 to 0.06 cc of heptaldehyde, dissolved in the

2 Weber, J., Irwin, M. H., and Steenbock, H., *Am. J. Physiol.*, 1939, **125**, 593.

3 Shimotori, N., Emerson, G. A., and Evans, H. M., *J. Nutrition*, 1940, **19**, 547.

4 Kudrjashov, A. B., *Arch. exp. Path. Pharm.*, 1933, **169**, 275; Kudrjashov, B. A., and Benjajeva, H., *ibid.*, 1934, **175**, 489; Kudrjashov, B. A., and Agatov, P. A., *Ginekol. i. Akush.*, 1935, **6**, 1.

5 Kudrjashov, B. A., *Bull. biol. med. expt., U.R.S.S.*, 1938 **6**, 220; through *Chem. Abs.*, 1939, **33**, 3428.

6 Carruthers, C., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 336.

ethyl esters of lard fat acids, and beginning as late as the 13th day of gestation. In many cases the placental sign appeared early.

Our own observations on about 75 rats can be summarized briefly by saying that it has not been possible, without inflicting systemic damage or severe intoxication, to interrupt pregnancy in animals on an adequate diet (Purina dog chow) by administration of products of fat oxidation, subcutaneously, intraperitoneally, or by mouth, or by prolonged feeding of this diet after treatment with ethereal ferric chloride or other agencies designed to promote oxidative rancidity. Among the oxidation products administered by mouth were: 200-450 mg heptaldehyde in methyl oleate or olive oil daily for 18 to 21 days during pregnancy (1 cc of heptaldehyde daily for several days proved highly toxic); 0.5 cc biacetyl in cod liver oil for a like period. Many more substances when tested by subcutaneous (S) or intraperitoneal (I) injection failed to interrupt pregnancy: 50 mg of heptaldehyde in methyl oleate (I) daily for 20 days; 1 cc (S) of heptaldehyde or pelargonic aldehyde on the 6th, 7th and 8th days of pregnancy; (very deep and severe lesions appeared at the site of injection); 0.5 cc to 1 cc doses (S) of irradiated unsaponifiable matter of cod liver oil, irradiated rancid lard or cod liver oil, their unsaponifiable matter, and the highly pro-oxygenic volatile products obtained during the oxidation of oleic acid or of aerated lard,[7] given daily between the 4th and 8th day of pregnancy, sometimes earlier or later. None of these measures prevented the gestation from being completed, unless the doses were so large or so often repeated as to cause the death of the animal. The mortality of the young was high.

The presence of 0.75 to 2% of heptaldehyde in the stock diet over long periods did not interrupt gestation; some of these animals were later subjected to the tests described above. More surprising was the capacity of ferric chloride-ether treated dog chow (first ground to powder) to maintain reproduction; this diet was begun with some young animals, was continued for many days (over 100) and was even successful in the second generation. The presence of additional cod liver oil during the spontaneous evaporation of the ether had no apparent effect.

One explanation for the lack of agreement between these results and those of the Russian investigators would appear to reside in differences in body stores; some of the variability revealed by their protocols suggests that their animals had been on a borderline diet, with a resulting confusion such as has occurred before in the history

[7] Deatherage, F. E., and Mattill, H. A., *Ind. Eng. Chem.*, 1939, **31**, 1425.

of vitamin E.[8] The effect of these pro-oxidants may not be entirely nil in the rat, but it has not been confirmed in our normally nourished animals.

The innocuous character of non-toxic doses of heptaldehyde with respect to reproduction in rats recalls similar observations of the Wisconsin investigators.[2] The effectiveness of small doses in abolishing reproduction in mice, even when pregnancy is far advanced, indicates a much greater susceptibility in these small animals than in rats. Effective total injections in mice[6] ranged from 0.04 to 0.38 cc, whereas 3 cc and, exceptionally, even 5 cc failed to interfere with reproduction in rats if they survived. Carruthers[9] has confirmed the resistance of rats to this treatment. On the other hand, Bryan and Mason[10] in a reëxamination of the vitamin E requirements of the mouse found male mice fertile even after 400 days on an E-deficient diet that produces sterility in male rats at 100 to 150 days.

The reproductive behavior of rats on ferric chloride-treated dog chow is further evidence of the inadequacy of this treatment completely to rid a diet of its vitamin E content. For a coupled reaction requiring the simultaneous oxidation of fat, intimate contact of the fat and ferric chloride must be secured for a sufficient length of time; aqueous ferric chloride was not effective[5] and possibly the character of the diet might provide certain physical barriers that would prevent adequate contact.

Summary. It has not been possible to interfere with the process of reproduction in female rats on an adequate diet by administering various oxidation products of fats, by mouth, subcutaneously, or intraperitoneally. These products included rancid animal fats, their volatile oxidation products with extremely high peroxide content, the unsaponifiable portion of irradiated fats and some aldehydes. Mortality of the young was very great. Unlike mice, rats were not susceptible to the damaging effect of heptaldehyde on reproduction; unless the mothers succumbed to systemic intoxication they bore litters even with serious lesions at the site of injection. An adequate stock diet (Purina dog chow, finely ground) treated with ethereal ferric chloride supported reproduction in the second generation, indicating that the coupled oxidation of tocopherol in the presence of rancid fats requires adequate contact for an adequate time.

[8] Mattill, H. A., *Am. J. Physiol.*, 1927, **79**, 305; Evans, H. M., *J. Nutrition*, 1928, **1**, 1.

[9] Carruthers, C., personal communication.

[10] Bryan, W. L., and Mason, K. E., *Am. J. Physiol.*, 1940, **131**, 263.

12006 P

Relative Metabolic Activities of Normal and Tumorous Liver Nucleoproteins Indicated by Radiophosphorus.*

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The observations that tumors are high in arginase[1] and mono- and polynucleotidase[2] activities, and recent studies with radiophosphorus,[3, 4] indicate rapid metabolism of the nuclear substance of neoplastic tissues. However, in these experiments, except those comparing lymphoma with normal lymph node,[4] it has been necessary to contrast cancer cells with various kinds of normal cells of different types. The liver carcinoma which can be produced by feeding azo dyes[5] is admirably suited to such experiments since the analogous normal tissue is readily available.

In Experiment I three groups of stock albino rats were used, one receiving a semi-synthetic diet (dextrin 79, casein 10, butter 5, salts 4, yeast 1, cod liver oil 1), another receiving in addition 0.06% dimethylaminoazobenzene (DAAB), and the third receiving 10% whole dried liver in addition to the basal ration and the azo dye. In Experiment II two groups of stock albino mice were used, one receiving a stock diet,[6] and the other in addition 0.05% p-aminoazotoluene (AAT). The rats were kept on the diet for 5 months and the mice for 7 months before the experiments, at which time all the animals receiving the azo dyes but no liver had developed hepatomata. Of 10 rats receiving both the azo dye and whole liver, only one developed liver tumors, and was discarded.

Each animal received subcutaneously 0.5 cc of a neutral radiophosphate solution. After 24 hours the animals were killed and the livers extracted 2-3 times with cold 0.01-2 N NH_4OH. The entire protein fraction was precipitated with cold trichloracetic acid, and

* We are indebted to the Jonathan Bowman Cancer Fund and the Wisconsin Alumni Research Foundation for financial support, to Dr. J. L. McKibben of the Physics Department for the supply of radiophosphorus, and to Dr. W. L. Van Horne of the Chemistry Department for the counting circuits.

1 Edlbacher, S., and Merz, K. W., *Z. physiol. Chem.*, 1927, **171**, 252.

2 Edlbacher, S., and Kutscher, W., *Z. physiol. Chem.*, 1931, **199**, 200.

3 Marshak, A., *Science*, 1940, **92**, 460.

4 Tuttle, L. W., Erf, L. A., and Lawrence, J. H., *J. Clin. Invest.*, 1941, **20**, 57.

5 Kinoshita, R., *Trans. Soc. Path. Japan*, 1937, **27**, 665.

6 Steenbock, H., *Science*, 1923, **58**, 449.

the precipitate washed several times each with cold dilute trichloracetic acid, acetone, alcohol at 70°, and ether, in order to remove all non-nucleic phosphorus. The residue was then wet-ashed, the total phosphorus determined colorimetrically, and the radioactivity measured with a Geiger-Müller counter.

For each animal we have calculated the fraction of the activity administered per gram of animal which was found per milligram of phosphorus in the isolated fraction. The results (Table I) show that the tumorous livers exhibit an increased uptake of labeled phosphate in the nucleoprotein fraction compared to the controls. This increase amounted to 44 ± 11% with the rats and 46 ± 7% with the mice. If the increased activity resides only in the cancer cells, the actual activity of the latter must be considerably greater than that observed, since they constitute only a fraction of the liver bulk. The increased activity of the liver-fed group (22 ± 7%) is probably due in part to the better health and food consumption of these animals and in part to their fatty condition which caused their weights to be high without a correspondingly high phosphorus content.

Since these experiments provide a comparison between normal and cancer cells derived from the same primordial cells and located in the same part of the body, they indicate that the nucleoprotein metabolism is increased in neoplastic tissue.

TABLE I.
Twenty-four-hour Uptake of Radiophosphorus by Normal and Tumorous Liver Nucleoproteins of Rats and Mice.

Exp. animals	I. Rats			II. Mice	
P^{31} inj.	.015 mg			.065 mg	
P^{32} inj.	35,000 counts/min			25,000 counts/min	
Diet	Semi-synth.	Semi-synth. + DAAB	Semi-synth. + DAAB + 10% liver	Stock	Stock + AAT
Avg wt (g)	258	146	318	20.6	24.2
Condition of livers	Normal	Tumorous	Normal	Normal	Tumorous
Activity of liver nucleoprotein	.179	.250	.272	.081	.128
	.205	.253	.275	.104	.132
	.223	.287	.280	.108	.156
	.234	.297	.286	.112	.160
	.236	.304	.286	.116	.160
	.275	.319	.300	.122	.161
	.309	.353*	.302		.161
		.400	.316		.167
		.423			.175
		.427*			
Avg activity	.237	.341	.290	.107	.156
Standard error	± .016	± .021	± .005	± .006	± .005

*Avg value for 2 rats whose liver nucleoproteins were accidentally mixed. Weight = 2.

12007

Presence of a Factor in Blood Which Enhances Bacterial-Growth Activity of Riboflavin.*

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Previous to 1939, the two principal methods of determining the content of riboflavin in biological materials were the rat growth method and some method which utilized the fluorescent properties of riboflavin. The first method had the disadvantages of being inapplicable for substances that contained very small amounts of riboflavin and of being cumbersome, time-consuming, and expensive. The second method was complicated by the presence of other pigments and by the uncertainty as to whether the fluorescent substance determined was in reality physiologically active riboflavin or some photoderivative of riboflavin. As a consequence, determination of riboflavin in the blood was impractical by either of these methods. It was, therefore, with considerable interest that the microbiological assay of Snell and Strong[1] was noted. Not only was this method applicable to substances containing minute amounts of riboflavin, but also it was quick and apparently specific for physiologically active riboflavin, since photoderivatives of riboflavin have no effect on the growth of bacteria. It was not long until the method had been applied to animal and human blood with fair success[2] and later with apparently complete success.[3]

It was our belief, however, that the specificity of the method had not been sufficiently proved to permit its application to such a complex substance as blood. Accordingly, we devised a method whereby

* We wish to express our gratitude to the Department of Bacteriology, Western Reserve University School of Medicine, for kindly placing the facilities of their department at our disposal for this work.

† S.M.A. Corporation Fellow and George Angell Fellow for research in Ophthalmological Chemistry.

1 Snell, E. E., and Strong, F. M., *Ind. and Eng. Chem.* (Analyt. Ed.), 1939, **11**, 346.

2 Fraser, H. F., Topping, N. H., and Isbell, H., *Pub. Health Rep.*, 1940, **55**, 280; Feeney, R. E., and Strong, F. M., *J. Biol. Chem.*, 1940, **133**, xxxi; Strong, F. M., Feeney, R. E., Moore, B., and Parsons, H. T., *J. Biol. Chem.*, 1941, **137**, 363.

3 Spies, T. D., Stanbery, S. R., Williams, R. J., Jukes, T. H., and Babcock, S. H., *J. A. M. A.*, 1940, **115**, 523; Spies, T. D., Bean, W. B., Vilter, R. W., and Huff, N. E., *Am. J. M. Sc.*, 1940, **200**, 697.

the riboflavin in the blood was destroyed by irradiation under alkaline conditions, the blood was then neutralized, and known amounts of riboflavin were added to it. This note reports the results of that work.

Methods. Human venous blood was collected and citrated. It was laked by the addition of 9 volumes of water, the pH was adjusted to 9 or 10 with NaOH, and the blood was then irradiated for from 10 to 12 hours under a 300 Watt incandescent bulb at a distance of about 6 inches. For the irradiation, the laked blood was placed in the inner tube of a condenser, through the jacket of which water was circulated to prevent heating. Following irradiation, the blood was neutralized with 10% HCl to a pH of 6.8 to 7.0. This irradiated blood was then used in the following manner:

Fifty tubes were prepared, each of which contained 5 cc of the basal medium of Snell and Strong.[1] To one group of tubes, pure riboflavin in amounts of 0.0, 0.05, 0.075, 0.10, 0.15, 0.20, 0.25, and 0.40 gamma was added and the contents of the tubes were diluted to 10 cc. To the other tubes, 1 cc or 2 cc of the irradiated blood (1:10) was added, then riboflavin in amounts of 0.0, 0.02, 0.04, 0.06, 0.08, 0.10, 0.15, 0.20, 0.25, and 0.40 gamma was added, and the mixtures were diluted to 10 cc. All of the tubes were then sterilized in an autoclave for 15 minutes at 15 pounds pressure. When cool, each of the tubes was inoculated with 0.15 cc of the inoculum. The inoculum was prepared by centrifuging a 24-hour growth of *Lactobacillus casei* ϵ‡ in the basal medium to which one gamma of pure riboflavin had been added. The cells were washed with 10 cc of 0.9% sterile saline solution, centrifuged, and resuspended in 4 cc of 0.9% sterile saline solution. One cc of this suspension was added to 100 cc of sterile saline solution, and this suspension was used for inoculating. This inoculum was adopted at the suggestion of Dr. M. Landy.[4]

All tubes were incubated for 72 hours at 37.5° C. They had been thoroughly shaken immediately following inoculation and were shaken again at 24 and 48 hours. The tubes were removed from the incubator, and those that contained the irradiated blood were centrifuged, the precipitate was washed once with 7 cc of distilled water, and the supernatant fluid titrated with 0.1 N NaOH to a definite blue end point with 6 drops of brom thymol blue (0.04%) as an indicator.

‡ The original culture of this organism was obtained from Dr. F. M. Strong of the University of Wisconsin.

[4] Landy, M., personal communication.

Results. A typical example of the results is given in Fig. 1. These results have been duplicated in 6 experiments with different samples of blood. It can be seen that the amount of acid produced by *L. casei* is much greater when irradiated blood is added to the basal medium than would be expected from the amount of riboflavin which had been added. That this growth is not due to the presence in the blood of riboflavin that has not been destroyed by irradiation is evidenced by the negligible growth obtained when irradiated blood alone was added to the basal medium. On the other hand, 1 cc of non-irradiated blood produces about 1.8 cc of acid, 2 cc about 2.5 cc of acid, and 4 cc about 4.5 cc of acid.

Comment. From these results, it is evident that blood contains some substance which, by itself, in the absence of riboflavin, is without effect upon the acid production by *L. casei* but which stimulates

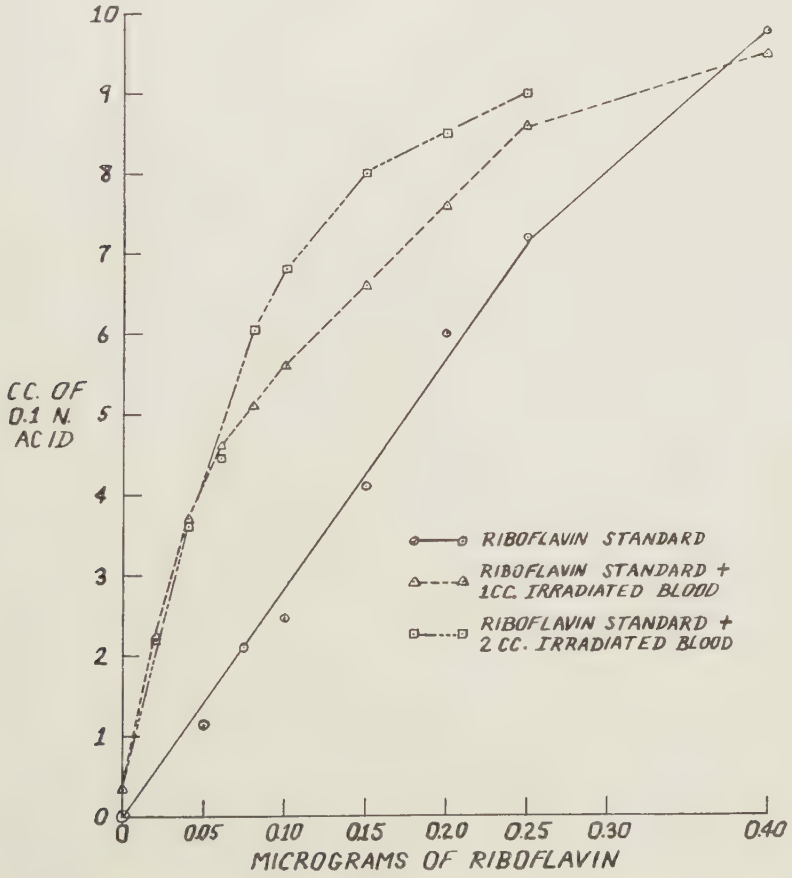

FIG. 1.

the growth of this organism in the presence of riboflavin. Preliminary studies indicate that this substance is not biotin, for a relatively pure biotin solution added to the basal medium in amounts of 2.5 rat units per tube produced no such effect. Furthermore, it seems unlikely that it is any known component of the vitamin B_2 complex, since doubling the amount of yeast extract produced no similar effect. This yeast extract should contain all the known components of the B complex except biotin and riboflavin, which were adsorbed to the lead sulphide in the course of preparation.[1]

Citrated plasma apparently contains an equal quantity of the factor, for plasma irradiated in the same way as the whole blood exhibited the same effect. Blood treated with Tonsil,§ which is believed selectively to adsorb the riboflavin, behaves like irradiated blood. However, 2 cc of Tonsil-treated blood give the same results as 1 cc of irradiated blood, suggesting that the factor may be partially adsorbed by the Tonsil. These experiments with Tonsil further suggest that the factor is not a substance produced by the irradiation process. On the other hand, breast milk treated with Tonsil‖ and urine irradiated for 4 hours under alkaline conditions do not exhibit the same effect.

It is also apparent that substances to be assayed for riboflavin should first be tested to see whether or not this factor is present, by destroying the riboflavin, either by irradiation or by Tonsil treatment, and by then adding known amounts of riboflavin to them. Unless similar tests are performed, the determinations made by this method are of questionable value because of the possibility of the presence of this factor. Similarly, the values for the content of riboflavin in the blood which have thus far been reported in the literature represent not the true value of riboflavin in the blood but only an apparent value. Blood values must be re-determined by using as a standard curve a basal medium to which riboflavin-free blood, in amounts equivalent to those being used for assay, has been added before the riboflavin is added. The significance attached to variations in the riboflavin content of blood before and after treatment must now be reconsidered.

§ Tonsil is a special fuller's earth which may be obtained from L. A. Salomon and Bros., 216 Pearl Street, New York, N. Y.

‖ At our suggestion, Dr. M. Landy of the Research Laboratories of the S.M.A. Corporation, Chagrin Falls, Ohio, repeated our original observation on irradiated blood. Furthermore, he tested the effect of Tonsil treatment of blood and breast milk, and kindly supplied us with a copy of his data for comparative purposes. Our experiments with Tonsil were undertaken at his suggestion.

Conclusions. Human blood from which the riboflavin has been removed by irradiation contains some factor which enhances the growth-promoting (acid production) activity of riboflavin on *Lactobacillus casei* ϵ. In the future, investigators who make determinations of the riboflavin content of blood or other biological materials must take into consideration the presence or absence of this factor.

12008 P

Effects of Sex and Gonadotropic Hormones on Red Cell Counts of Rats.

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In a previous communication,[1] we have reported that normal female rats, injected for 2-3 months with pregnant mare serum hormone showed an erythrocyte count well below normal. Since hypophysectomy is also followed by an anemia,[2] it seemed to us that a broad investigation of sex and gonadotropic effects on the blood picture might serve to clarify the so-called hemotropic action of the anterior pituitary.

In the first series of experiments, determinations were made upon groups of normal and castrated animals injected daily with different hormone preparations.* The red cell counts of 47 normal mature rats of our colony were found to vary from approximately 8.0-9.0 million per mm^3, and averaged about 8.5 million per mm^3. Eight normal females injected daily for 2 months with 15 r.u. Gonadin revealed an average red cell count of 7.5 million per mm^3. The average red cell count of 11 normal males treated for 2 months with 10-15 r.u. Gonadin was 10.1 million per mm^3. Eleven untreated castrate females, 2 months after the operation possessed an

1 Gordon, A. S., Levenstein, I., and Charipper, H. A., *Proc. Am. Physiol. Soc.*, 1940, 68.

2 Vollmer, E., Gordon, A. S., Levenstein, I., and Charipper, H. A., *Endoc.*, 1939, **25**, 970.

* We wish to thank Dr. Erwin Schwenk, Schering Corporation, for generous supplies of testosterone propionate, estradiol benzoate, and Pranturon (a human pregnancy urine preparation). We are also indebted to Mr. Donald Wonder, Cutter Laboratories, for Gonadin (a pregnant mare serum preparation).

average count of 9.4 million per mm^3. Daily injections, for 2 months, of 5 r.u. estradiol into 5 castrated females lowered the red cell count to 7.8 million per mm^3. The counts of 5 castrated females, injected daily for 2 months with 0.5 mg testosterone averaged 9.8 million per mm^3. The red cell counts of castrated rats injected with Gonadin, sesame oil, or normal horse serum remained unaltered.

In the second series of experiments, hypophysectomized rats were employed. We have already shown[2] that complete hypophysectomy in rats is followed by a decline in red cell count which reaches 60-70% its normal value after about 2 months. Three females, hypophysectomized 6 weeks previously, showed no response to daily injections of 15 r.u. Gonadin (average, 6.1 million per mm^3), but 4 hypophysectomized males attained and, in 2 cases, surpassed the normal levels (average, 9.0 million per mm^3) at the end of one month of treatment. The same dosage of pregnant mare serum did not prevent 3 females from revealing the usual post-hypophysectomy anemia when injections were begun immediately following the operation. The average red cell counts of 3 hypophysectomized males and 3 hypophysectomized females, injected daily with control doses of normal horse serum (0.3 cc) were unchanged. Daily injections of 20 i.u. Pranturon were found to have little effect on the blood picture of 3 hypophysectomized males and 3 hypophysectomized females.

The most striking effects were obtained with male sex hormone. Six hypophysectomized females and one hypophysectomized male, injected daily with 1.0 mg testosterone (in sesame oil) showed gains of from 2-4 millions per mm^3 in one month. Testosterone injections, in most cases, evoked a reticulocytosis (as high as 10%). Hemoglobin values showed constant gains but did not reach normal levels. Injections of sesame oil were without effect.

Summary. 1. Pregnant mare serum hormone, when injected into normal and hypophysectomized males, causes an elevation in red cell count. Injections of testosterone into castrated females or hypophysectomized males and females likewise increase red cell count. 2. The red cell counts of normal females treated with pregnant mare serum hormone are lowered, those of castrate or hypophysectomized females, similarly treated, remain unchanged. Estradiol lowers the counts of normal females. 3. It seems possible that the gonadal secretions are responsible, to some extent, for the sex difference in normal red cell count encountered in many species of animals.

12009

Effect of Pituitary Growth Hormone on the Thymectomized Rat.*

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The problem of the relationship of the thymus gland to growth is one which has received considerable attention in the last decade. On the one hand, experimental work in the laboratories of Asher[1] and of Rowntree[2] employing thymus gland extracts has given presumptive evidence of a growth-promoting agent in the thymus gland. On the other hand, it has been observed in this laboratory[3] that growth hormone preparations usually cause enlargement of the thymus gland in both hypophysectomized and normal animals. Further, adrenocorticotropic hormone has been demonstrated to cause thymic atrophy while, at the same time, under certain experimental conditions, causing inhibition of somatic growth.[4] The question has arisen, then, as to whether or not the growth hormone is "thymotropic" and whether its effect in stimulating growth is mediated by the thymus gland. The experimental evidence presented in this paper attempts to answer the latter part of this question.

Effect of growth hormone on hypophysectomized-thymectomized female rats. Female rats of the Long-Evans strain thymectomized when 8 days of age and weaned at 21 days of age, were allowed to grow until 84 days of age at which time they were hypophysectomized. The animals were allowed to plateau after hypophysectomy until the age of 104 days when injections of growth hormone were begun and continued for 20 days (excluding Sundays) at which time the animals were autopsied. The animals were weighed twice weekly to the time of autopsy. Hypophysectomized littermates which had been subjected to a sham thymectomy at the same time as the other experimental animals were also injected with the same growth hormone preparation under the same experimental condi-

* Aided by grants from the Board of Research of the University of California and the National Research Council Committee for Research in Endocrinology.

1 Asher, L., *Endokrinologie*, 1930, **7**, 321.

2 Rowntree, L. G., Clark, J. H., Hanson, A. M., and Steinberg, A., *Arch. Int. Med.*, 1935, **56**, 1.

3 Fraenkel-Conrat, H. L., Meamber, D. L., Simpson, M. E., and Evans, H. M., *Endocrinology*, 1940, **27**, 605.

4 Crede, R. H., and Moon, H. D., Proc. Soc. Exp. Biol. and Med., 1940, **43**, 44.

tions. Hypophysectomized-thymectomized littermates were used as uninjected controls. The animals were fed this laboratory's Diet XIV† throughout their life supplemented by wet Diet I† during the period of injection. At autopsy, the sites of the pituitary and thymus were carefully searched for evidence of remaining fragments. Any suspicious fragments were subjected to microscopic examination to check completeness of operation. None of the animals reported showed incomplete operations.

The growth hormone employed was a "globulin" fraction,[5] prepared from the anterior lobes of beef pituitaries desiccated with acetone in the cold, extracted in alkaline solution, and precipitated with ammonium sulfate. The preparation contained adrenocorticotropic, lactogenic and smaller amounts of interstitial cell stimulating and thyrotropic hormones. The injected animals each received intraperitoneal doses of an amount of hormone represented by 200 μg of dry weight on the first day, 350 μg on the second and third days, and 500 μg daily from the fourth to the 20th day in 1.0 cc fluid. (The growth potency of the hormone preparation was 30 units[6] per milligram when assayed in other hypophysectomized rats tested concurrently with the experimental groups.)

The results in the experimental groups of animals are presented in Table I. The growth curves of all the animals were similar to the time of injection. It is significant that there was no difference in the growth of the thymectomized animals as compared to the sham operated littermate controls.

The evidence presented indicates that in hypophysectomized animals growth hormone is equally effective either in the absence or presence of the thymus gland.

Effect of growth hormone on adult thymectomized female rats with intact pituitaries. In the second part of this experiment it was proposed to determine whether or not thymectomy would influence the response of plateaued female rats (with intact pituitaries) to a

† Diet I: 67.5% whole wheat (ground); 15% casein; 10% whole milk powder; 1% sodium chloride; 1.5% calcium carbonate; 5% milk fat (melted butter).

Diet XIV: 67% whole wheat (ground); 5% fish oil; 5% casein; 10% alfalfa leaf meal; 10% fish meal; 3% sodium chloride supplemented with fresh lettuce twice weekly.

5 Marx, W., The method, to be published in detail, is a modification of the procedure published in 3.

6 Evans, H. M., Uyei, N., Bartz, Q. R., and Simpson, M. E., *Endocrinology*, 1938, **22**, 483. A unit is defined as the amount of a growth hormone preparation which causes an average body weight gain of one g per day (10-day test, hypophysectomized rat).

TABLE I.

Type of animal and treatment	No. of animals	Avg initial B.W.(g)	Gain in B.W. (g)*		Avg gain in % of initial B.W.
Hypophysectomized, treated with growth hormone	4	155	50 43 53 50	*49*	32
Hypophysectomized—thymectomized, treated with growth hormone	6	153	34 30 52 50 52 36	*42*	28
Hypophysectomized—thymectomized, untreated	5	149	—4 0 3 —3 4	*0*	0

*Figures in italics represent the average weight gain of the group.

potent growth hormone preparation. Female rats thymectomized at the age of 8 or 9 days and weaned at 21 days of age were allowed to grow until the usual plateauing of the growth curve had occurred. At the age of about 6 months, the animals were injected with the same preparation of growth hormone, receiving daily intraperitoneal injections of 1.0 cc solution containing 2.5 mg of the preparation for 20 days (excluding Sundays). The animals were weighed weekly until the time of injection and twice weekly during the time of injection. Thymectomized and sham operated littermates served as untreated controls and in addition one group of sham operated littermates was injected with the same amount of growth hormone to determine any difference in response which might occur depending on the presence or absence of the thymus gland. At autopsy, all suspicious tissue in the neck regions of the thymectomized animals was subjected to microscopic examination to check the completeness of operation. In no case in the groups reported was a fragment of thymus gland found in the normal position. A few of the animals suffered from superficial wound infections during the first postoperative week. The growth of these animals did not differ from that of their controls.

The growth curves of all the animals were similar, there being no significant difference up to the time of growth hormone injection between the thymectomized animals and their sham operated littermate controls. The response of these groups to the injection of growth hormone is summarized in Table II.

As indicated, there is no significant difference in growth either in

TABLE II.

Type of animal and treatment	No. of animals	Avg initial B.W. (g)	Gain in B.W. (g)*	Avg gain in % of initial B.W.
Normal control, untreated	5	256	33 26 *24* 26 24 11	9
Thymectomized, untreated	7	238	28 18 *26* 32 18 17 26 42	11
Normal, treated with growth hormone	6	225	44 63 *57* 54 68 68 47	25
Thymectomized, treated with growth hormone	8	239	60 50 *64* 45 49 71 81 61 98	27

*Figures in italics represent the average weight gain of the group.

the treated or untreated groups when the thymectomized animals are compared with their controls. The increase in weight of the control groups after a time in which they were plateaued may, in all probability, be ascribed to the change in environment and diet. At the beginning of the experimental period of growth hormone injection the diet of all animals was changed from Diet XIV to a combination of Diet XIV and Diet I, and the animals were moved to a different location in the colony.

Discussion. The experimental evidence presented allows one to state that under the conditions of the experiment, the presence of the thymus gland is not essential to the normal growth in body weight of the female rat in the time limits employed (8 days to 6 months). Nor is the thymus gland necessary for the marked increase in body weight produced by a potent growth hormone preparation when injected into female rats either in the presence or absence of the animal's own pituitary gland. The evidence presented here does not bear on the claim that one may extract from the thymus gland a substance or substances possessing growth promoting properties.

Whatever these may be, it is evident that they have not played a detectable rôle in the experiments here reported.

Summary. 1. The growth in body weight of female rats thymectomized at the age of 8 days and followed to the age of 6 months is the same as that of sham operated littermate controls. 2. The response of hypophysectomized-thymectomized female rats to a preparation of the pituitary growth hormone is the same as that of similarly treated hypophysectomized littermate controls which had been subjected to a sham thymectomy. 3. The response of plateaued thymectomized female rats with intact pituitaries to a potent growth hormone preparation of the anterior pituitary is the same as that of sham operated littermate controls. 4. Under the conditions of this experiment, therefore, the thymus gland was not necessary either for the growth in weight of otherwise normal animals or for the marked increase in body weight produced by the administration of anterior pituitary growth hormone.

12010

Absorption of 2-Methyl-1,4-Naphthoquinone and Phthiocol by Bile Fistula Rats.*

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It is now well established that deoxycholic acid is necessary for the absorption of vitamin K. The fact that certain water soluble compounds possess vitamin K activity has raised the question as to whether or not these substances are absorbed from the intestinal tract in the absence of bile. Smith and Owen[1] have shown that oral administration of 4-amino-2-methyl-1-naphthol to patients suffering from chronic obstructive jaundice led to an increase in the level of prothrombin. Similar results were reported by Warner and Flynn[2] on administration of the potassium salt of the disulfuric acid ester of 2-methyl-1,4-naphtho-hydroquinone to vitamin K depleted bile-

* Aided by grants from the Christine Breon Fund of the University, and Eli Lilly and Company. Technical assistance was supplied by W.P.A. project No. 65-1-08-62 (unit A-12). The compounds tested were kindly supplied by Dr. H. J. Almquist.

1 Smith, H. P., and Owen, C. A., *J. Biol. Chem.*, 1940, **134**, 783.

2 Warner, E. D., and Flynn, J. E., Proc. Soc. Exp. Biol. and Med., 1940, **44**, 607.

obstructed rats. The present experiments are in harmony with these two observations, although the compounds used and the experimental technic employed were somewhat different.

Choledochocolonostomized rats were maintained on a low vitamin K diet and were kept in cages with wide mesh screen bottoms to prevent their having access to their feces. Three to 6 weeks following the operation there was a decrease in the prothrombin content of the blood. For the prothrombin estimations, blood was obtained by heart puncture. In this connection it should be pointed out that it is not feasible to obtain blood in this manner when the prothrombin levels are much lower than about 50%, due to the possibility of hemorrhage. The prothrombin levels were estimated according to the method of Almquist and Klose,[3] modified so that only 0.5 cc of blood was required. When the prothrombin level had fallen to about 50%, the compound under test was administered orally. The data are given in Table I.

The data indicate that both phthiocol and 2-methyl-1,4-naphthoquinone are absorbed from the intestinal tract of bile fistula rats, and that deoxycholic acid is not necessary to insure the absorption of these compounds. Failure of deoxycholic acid alone to lead to an increase in prothrombin is due to the absence of vitamin K in the diet. Unless the test substances are administered at intervals of several days, the prothrombin levels in the experimental rats return to the low levels.

The experiments indicate the feasibility of using the compounds tested orally in order to raise the prothrombin level of the blood.

TABLE I.

Absorption of Phthiocol and 2-Methyl-1,4-Naphthoquinone by Bile Fistula Rats.

Substance administered	No. of animals	Avg No. of days after operation	Avg prothrombin level before test, %	Avg prothrombin level after test, %
I	8	38	52	100
II	8	32	54	100
III	7	35	55	100
IV	8	33	50	100
V	8	31	54	54

I. Each animal given 1 mg of 2-methyl-1,4-naphthoquinone orally.
II. Each animal given 1 mg of the choleic acid of 2-methyl-1,4-naphthoquinone prepared according to Almquist and Klose[4] orally.
III. Each animal given 1 mg of 2-methyl-1,4-naphthoquinone plus 5 mg of deoxycholic acid orally.
IV. Each animal given 1 mg of phthiocol orally.
V. Each animal given 5 mg of deoxycholic acid orally.

[3] Almquist, H. J., and Klose, A. A., *Biochem. J.*, 1939, **33**, 1055.

[4] Almquist, H. J., and Klose, A. A., *J. Am. Chem. Soc.*, 1939, **61**, 745.

12011

Aerobic Fat Metabolism of *Ascaris lumbricoides.*

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In previous experiments[1] it was shown that *Ascaris,* during a starvation period of 24 hours, consumes a large amount of carbohydrate and a small amount of protein, but no fat. Mueller,[2] however, found that all the morphologically demonstrable fat disappears from explanted pieces of *Ascaris* tissue in 8 days, and Hirsch and Bretschneider[3] observed that during starvation much stainable fat disappears from the intestinal cells of this parasite. It seemed of interest to investigate the fat changes in *Ascaris* during a longer starvation period with a chemical method, since so far no convincing evidence is available as to whether any parasitic nematode is able to consume fat.

Consequently a series of experiments was performed in which, on the one hand, the fat content of freshly collected females of *Ascaris lumbricoides* from the pig was determined according to the method of Kumagava and Suto,[4] while the corresponding experimental worms, on the other hand, were kept for 5 days in 1% saline at 37°C under aerobic conditions. These latter worms were weighed individually at the beginning of the experiments and kept in separate containers, since out of a given lot of worms some always will die under these conditions. Moreover, it seemed essential to know the exact weight of the worms at the start of the starvation period. The saline was changed every 24 hours and the eggs discharged into it were collected individually for every worm by centrifugation. At the end of the starvation periods the surviving worms were used for a determination of the ether extract, and, in a separate analysis, the fat content of the discharged eggs was determined. A total of 12 experiments was performed with 230 worms, about equally distributed between worms analyzed at the beginning and after starvation.

The figures given below for the fat percentage of starved worms

1 v. Brand, Th., *Z. vergl. Physiol.,* 1934, **21**, 220.

2 Mueller, J. F., *Z. Zellfschg.,* 1928, 361.

3 Hirsch, G. Ch., and Bretschneider, L. H., *Cytologia* (Tokyo), 1937, Fujii Jub. Vol., 424.

4 Kumagava, M., and Suto, K., *Bioch. Z.,* 1908, **8**, 128.

are calculated on the basis of the weight of the worms at the beginning of the starvation period. This is the only way of assuring an accurate comparison between the fat content of worms analyzed at the beginning of the experiments and animals kept for some time *in vitro,* since the weight of ascarids kept for some days in saline shows a tendency to increase somewhat due to water intake, a factor which of course had to be eliminated in order to allow comparisons on a percentage basis.

The freshly collected worms contained $1.75 \pm 0.07\%$ ether extract; the body of the starved worms contained $1.67 \pm 0.05\%$, while the ether extract of the discharged eggs corresponded to $0.05 \pm 0.013\%$ of the weight of the worms. The sum of the ether extracts derived from the body and the discharged eggs of the experimental worms was therefore practically identical with that found at the beginning, indicating that no noticeable amount of fat had been used for production of energy. The amount of fat due to discharged eggs was relatively high and would probably have been higher under natural conditions. Fauré-Fremiet[5] has shown that *Ascaris* eggs contain a fatty compound (ascaryl alcohol) that certainly does not occur in the food of the worms and which, therefore, must be synthesized from other compounds. These observations seem to justify the assumption that *Ascaris* has a fat metabolism of some intensity which, however, is not connected with energy production, at least not during a starvation period of 5 days. The cited observations of Hirsch and Bretschneider may show a step in these synthetic processes. Mueller's findings, if corroborated by chemical analysis, would indicate, however, that the metabolism of explanted tissues of *Ascaris* must be different from that found in intact worms.

Summary. Females of *Ascaris lumbricoides* excrete in their eggs during a 5 days' starvation period at 37°C under aerobic conditions, a noticeable amount of ether soluble material, indicating the presence of a fat metabolism of some intensity. The sum of the ether extracts derived from the body and the eggs of the starved worms corresponds closely to that found in unstarved worms, showing that during the starvation period no fat had been used for the production of energy.

[5] Fauré-Fremiet, E., *Arch. d'Anat. microsc.,* 1913, **15**, 435.

12012 P

Electrophoretic Behavior of the Papilloma Virus Protein.*

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In 1937 a specific heavy protein was isolated[1] by differential ultracentrifugation from infectious extracts of cottontail-rabbit papillomas.[2] Purified by several cycles of alternate low- and high-speed ultracentrifugation the material sediments in the analytical ultracentrifuge with the sharp boundary characteristic of a single molecular species and with a sedimentation constant of $S_{20}^{\circ} = 265 \times 10^{-13}$ cm sec^{-1} $dynes^{-1}$. Studies on infectivity,[3] complement-fixing capacity,[4] and neutralization[5] with specific immune sera have demonstrated a high degree of uniformity in the biological behavior of the protein and its intimate relation to the papilloma virus. In the present work, further evidence of the homogeneity of the macromolecular protein is furnished by examination of its electrophoretic behavior.

The studies were made on about 150 mg of protein freshly prepared from 350 g of warts from many cottontail rabbits, extracted and purified by 3 ultracentrifugal cycles by the routine procedure previously described.[1] The purified product was dissolved in buffer solution consisting of 0.05 M sodium chloride and 0.05 M sodium veronal-sodium acetate to give a solution of 0.1 ionic strength and adjusted to the required pH with NaOH or HCl. Studies were made on dialyzed solutions at protein concentrations of 4.5 to 2.7 mg per cc. The instrument† employed was of the Tiselius[6] type. Movement of the protein boundary in the 11 cc cell under the influence of constant electric potential at 0°C and changes

* This work was aided by a grant from Lederle Laboratories, Pearl River, N.Y., and by the Dorothy Beard Research Fund.

1 Beard, J. W., Bryan, W. R., and Wyckoff, R. W. G., *J. Infect. Dis.*, 1939, **65**, 43.

2 Shope, R. E., *J. Exp. Med.*, 1933, **58**, 607.

3 Bryan, W. R., and Beard, J. W., *J. Infect. Dis.*, 1939, **65**, 306.

4 Bryan, W. R., Beard, D., and Beard, J. W., *J. National Cancer Institute*, in press.

5 Bryan, W. R., and Beard, J. W., *J. Infect. Dis.*, in press.

† We are greatly indebted to Dr. L. G. Longsworth, Rockefeller Institute for Medical Research, for his generous advice in the construction and operation of the instrument employed in these studies.

6 Tiselius, A., *Trans. Faraday Soc.*, 1937, **33**, 524.

in boundary shape were recorded photographically by the refractive index method of Svensson.[7]

The pH regions available for study of the intact protein are determined[8] by the limits of molecular stability in both acid and alkaline ranges and by insolubility near the isoelectric point. An example of the results obtained in the acid range of solubility is shown in Fig. 1 in the series of curves photographed after successive time intervals over a period of 5.5 hours at pH 3.78. The abscissas represent distances in the direction of migration of the protein boundary into protein solution while the ordinates are proportional to the refractive index gradient or protein concentration gradient in the

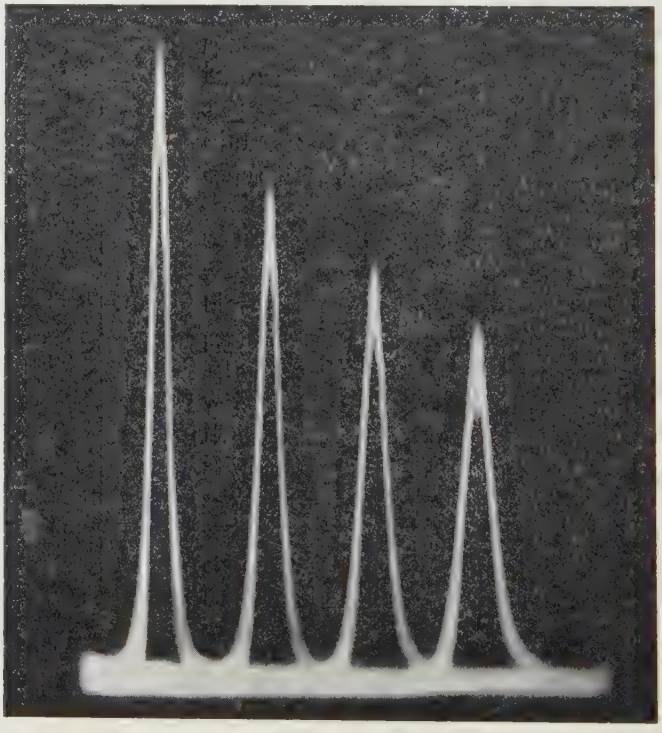

FIG. 1.

Svensson diagrams made with the rabbit papilloma virus protein. The initial peak was photographed at the beginning of the experiment; the succeeding peaks were photographed at intervals of 110 minutes for 5½ hours. The electric field was 3.26 volts/cm; the ionic strength, 0.1; and the pH, 3.78. The photographs record movement of the boundary (from left to right) into protein. The mobility was $+3.70 \times 10^{-5}$ cm²/sec./volt.

[7] Svensson, H., *Kolloid Z.*, 1939, **87**, 181.

[8] Beard, J. W., and Wyckoff, R. W. G., *J. Biol. Chem.*, 1938, **123**, 461.

boundary at that point. Throughout the experiment the boundary remained single and sharp, exhibiting no evidence of splitting. With continuous migration, slight spreading of the boundary occurred due to combined influence of diffusion and inhomogeneity of particle charge. At 0°C diffusion of the large molecules is very small and most of the spread is attributable to the latter factor. The extreme sharpness of the last single peak of Fig. 1 shows a remarkably high degree of uniformity of particle charge, indicative of corresponding homogeneity of the papilloma-virus protein in the acid range below the isoelectric point. Preliminary studies have been made in the region of solubility above the isoelectric point. Here, the protein moved with a single sharp boundary with slightly more spread and skewness in the final picture than in that of Fig. 1. In none of the studies thus far made (between pH 3.78 and pH 7.7) has there been any evidence of boundary splitting.

The results show that the papilloma protein is extremely well suited to studies in the Tiselius apparatus. Under the conditions described solutions of this animal virus protein exhibit on both sides of the isoelectric point a high degree of electrophoretic homogeneity comparable to or possibly greater than that of the most homogeneous hemocyanins[9] and crystalline proteins.[10] These findings combined with those of sedimentation studies provide a valuable criterion of the physical state of the material since the degree of homogeneity observed with the papilloma protein has been seen thus far only with solutions of a single molecular species.

12013

Additional Observations on Vitamin K-Deficient Diets.

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In recent papers Almquist[1, 2] emphasizes the precautions necessary in the care and housing of test animals in order to prevent bacterial

9 Tiselius, A., and Horsfall, F., Jr., *Arch. f. Kemi, Mineralogi och Geologi*, 1939, **13** A, 1.

10 Longsworth, L. G., Cannan, R. K., and MacInnes, D. A., *J. Am. Chem. Soc.*, 1940, **62**, 2580.

* Present address: International Vitamin Corporation, 50 East 42nd Street, New York City.

K-vitamin synthesis in wet feeds and in droppings, and states that heated diets[3] are not growth-promoting. These reports prompt us to present additional data on this type of diets.

Experiments conducted during the past months have shown that ration K-11 (Table I) containing p-aminobenzoic acid[4] is more suitable than the ration K-7, previously described[3] as being deficient in the rat anti-gray-hair factor, because it permits better growth of the test animals and an earlier incidence of the hemorrhagic diathesis. It was observed also that vitamin K is apparently a growth factor, since chicks lose weight when the deficient symptoms occur and grow at a greater rate on ration K-11 supplemented with vitamin K than on ration K-11 alone.

The type of casein in ration K-11 is an important factor. The SMACO† Vitamin-free Casein proved to be an excellent source of protein in a vitamin K-deficient diet, but Labco‡ Vitamin-free

TABLE I.
Vitamin K-deficient Ration K-11.*

		%
Heated grain mixture†	wheat middlings 25; yellow corn 58	83
Casein‡		12
Salt mixture[5]		2
Calcium carbonate		1
Cod liver oil (medicinal)		2

*Contains less than 0.1 μg of biotin per g and should, therefore, be supplemented with biotin, when the animals are not to be sacrificed at the termination of the vitamin K studies. Without added biotin, most of the chicks showed severe vitamin H-deficiency symptoms within 2 months and perosis was observed in about 20% of the birds at the end of the third month.

†A mixture containing 25 parts of wheat middlings and 58 parts of yellow corn heated to 120°C for one week.

‡A dilute ethanol solution containing

80 mg { thiamine hydrochloride, riboflavin, pyridoxine hydrochloride }

+

1 g { calcium pantothenate, nicotinic acid, inositol }

+

3 g { choline chloride, p-aminobenzoic acid }

is added to 1.2 kg of SMACO Vitamin-free Casein; the product is thoroughly mixed and then dried at a temperature not exceeding 70°C.

[1] Almquist, H. J., 56th Ann. Meet., A.O.A.C., Washington, D.C., October, 1940.

[2] Almquist, H. J., *Physiol. Rev.*, 1941, **21**, 194.

[3] Ansbacher, S., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 248.

[4] Ansbacher, S., *Science*, 1941, **93**, 164.

† Distributed by the General Biochemicals, Inc., Chagrin Falls, Ohio.

‡ Distributed by the Borden Co., New York.

Casein is apparently not entirely devoid of vitamin K, as the hemorrhagic diathesis does not develop in all the test animals reared on this diet in which this brand of casein is used as the basal protein. A similar observation has already been made in a comparative study of rations containing the Labco product and fish meal, respectively.[5]

The ration K-11 can be used even when wet. One hundred day-old chicks were placed on a diet consisting of ration K-11 thoroughly mixed with an equal part of fresh ripe bananas. All the birds on this diet showed the typical K-deficiency symptoms within the same period of time as the control birds on ration K-11. Apparently, bananas are devoid of vitamin K and do not contribute to a K-vitamin synthesis. This experiment shows also that ration K-11 is suitable for prophylactic bioassays necessary for the studies of substances with too low a vitamin K content for curative tests.

Every animal of 5 lots of 200 chicks each reared on ration K-11 became sufficiently vitamin K-deficient to be used for assays. The results obtained with some of these birds are recorded in Table II. The "Thyloquinone"§ employed was a corn oil solution containing 1 mg of 2-methyl-1,4-naphthoquinone per ml and the "Thylohydroquinone"§ was an isotonic solution containing 1 mg of 2-methyl-1,4-naphthohydroquinone per ml which had been stored in sealed ampoules at 105°F for a period of 3 months. The "Phosphate (I)" was the previously described product[6] and the "Phosphate (II)" was the preparation "N-123" of the Laboratories of Hoffmann-La Roche,‖ both phosphates presumably being sodium 2-methyl-1,4-naphthohydroquinone diphosphate.[7] The "Amino (2)" and the "Amino (3)" were the 2-methyl-4-amino-1-naphthol hydrochloride[8] and the 3-methyl-3-amino-1-naphthol hydrochloride,[8] respectively, both prepared by Parke, Davis Laboratories.¶ The "Quinoline-quinone" was the new compound 6-methyl-5,8-quinolinequinone recently described by Christiansen and Dolliver.[9]

The data of Table II confirm our previous results[6] that methyl-

[5] Ansbacher, S., *J. Nutrition*, 1939, **17**, 303.

§ Distributed by E. R. Squibb & Sons, New York.

[6] Ansbacher, S., Fernholz, E., and Dolliver, M. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 652.

‖ We wish to take this opportunity of thanking Dr. Foster for his kindness in sending us this preparation.

[7] Lee, J., Solmssen, U. V., Steyermark, A., and Foster, R. H. K., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 407.

[8] Emmett, A. D., Kamm, O., and Sharp, E. A., *J. Biol. Chem.*, 1940, **133**, 285.

¶ We wish to take this opportunity of thanking Dr. Kamm for his kindness in sending us these preparations.

[9] Christiansen, W. G., and Dolliver, M. A., *J. Am. Chem. Soc.*, 1941, **63**, in press.

TABLE II.
Vitamin K Activity Determined with Chicks on Ration K-11.

Substance	No. of animals used	Minimum effective dose in 6-hr test γ
Thyloquinone	154	½
Thylohydroquinone	233	½
Phosphate (I)	59	10
" (II)	23	1½
Amino (2)	26	1¼
" (3)	35	2
Quinoline-quinone	31	10 mg insufficient
Bananas (fresh, ripe)*	100	4 g daily insufficient

*Prophylactic test.

naphthoquinone and methylnaphthohydroquinone have identical biological activities. Apparently, solutions of the latter compound are stable even at elevated temperatures. In regard to the discrepancy in the potencies of the 2 phosphate derivatives, the Hoffmann-LaRoche preparation (Phosphate II) was found to be about as potent as methylnaphthoquinone on a molecular basis, thus confirming the recent data of Fieser, *et al.*[10] As suggested by Lee, *et al.*,[7] it may be that the Phosphate (I) had a low activity due to the fact that its preparation did not include the isolation and purification of the intermediate diphosphoryl chloride. The observation of Emmett, *et al.*,[8] that the amino compound with the methyl group in position 2 is more potent than the one with the methyl group in position 3, was confirmed. It is of interest to note also that the quinoline-quinone has apparently no antihemorrhagic activity.

12014

Blood Prothrombin Levels and Hippuric Acid Excretion Liver Function Test in Liver Disease.

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From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard School, Boston.

Wilson[1] showed that a close correlation existed between the hippuric acid excretion and the plasma prothrombin levels of 36 patients

[10] Fieser, L. F., Tishler, M., and Sampson, W. L., *J. Biol. Chem.*, 1941, **137**, 659.
[1] Wilson, S. J., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 559.

with liver disease whom he studied. He concluded that "the quantitative levels of plasma prothrombin and the amounts of hippuric acid excreted following the ingestion of a known quantity of sodium benzoate reflected most sensitively and consistently the degree of liver damage existing."

We have observed the hippuric acid excretion and the blood prothrombin level of 12 patients with clinically evident liver disease in the same way as Wilson did, save that in our patients these two tests were made *following adequate treatment with synthetic vitamin K analogues.*

In these 12 patients the "prothrombin time" was measured by the Quick technic[3] and converted to blood prothrombin percentage of normal.[4] The hippuric acid liver function test[2] was performed on the patients on the same day as the blood prothrombin level was estimated, or within the next 3 days.

The clinical diagnoses, response to therapy with vitamin K, final blood prothrombin level and hippuric acid excretion of these patients are recorded on Table I.

Conclusion. There was no correlation between the blood prothrombin level and a single estimation of the urinary excretion of

TABLE I.

Comparison of the Prothrombin Levels in Blood of Patients Suffering with Liver Disease (after Correction of Avitaminosis K) with Hippuric Acid Excretion, Measured at the Same Time.

Case No.	Clinical diagnosis	Response to therapy with vitamin K analogues	Blood prothrombin % of normal	Hippuric acid excretion, g
1.	Subacute yellow atrophy	Slight, delayed	35	2.62
2.	Portal ("alcoholic") cirrhosis	None	40	1.12
3.	" " "	"	40	1.2
4.	Catarrhal jaundice	Moderate, delayed	40	1.3
5.	Cardiac failure with hepatitis	" rapid	50	1.5
6.	" " " "	" "	58	1.1
7.	Portal ("alcoholic") cirrhosis	None	60	1.3
8.	" " "	"	65	<1
9.	Stricture of common duct. Biliary cirrhosis	Complete, rapid	100	<1
10.	Cancer of gall bladder. Biliary cirrhosis	" "	100	1.3
11.	Cancer of head of pancreas. Biliary cirrhosis	" "	100	0.43
12.	Recurrent jaundice. ?Toxic hepatitis. ?Obstruction	" "	100	1.2

[2] Quick, A. J., *Am. J. Med. Sc.*, 1933, **185**, 630.

[3] Quick, A. J., Stanley-Brown, M., and Bancroft, F. W., *Am. J. Med. Sc.*, 1935, **190**, 501.

[4] Souter, A. W., and Kark, R., *Am. J. Med. Sc.*, 1940, **200**, 603.

hippuric acid after ingestion of known amounts of sodium benzoate in 12 patients with liver disorders after treatment with synthetic vitamin K analogues.

12015 P

Etiological Agents of North and South American Blastomycosis.*

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A granulomatous fungus infection simulating scrofuloderma was described by Gilchrist in 1894. Since the fungus was seen in the diseased tissues as a budding, yeast-like organism, associated with a skin infection, it was called *Blastomyces dermatitidis*[1] and the disease *blastomycosis*. A number of different species of *Blastomyces*[2] and a number of variously named fungi[3] since reported from cases of North American blastomycosis have been shown to be either slight variations of Gilchrist's organism or to be *Coccidioides immitis*,[4, 5] the causative agent of San Joaquin Valley Fever or coccidioidal granuloma.

South American investigators have described a granulomatous fungus infection as pseudococcidioidal granuloma[6] caused by a fungus first confused with *Coccidioides immitis*,[7, 8] later named *Paracoccidioides brasiliensis*.[9] Moore,[10] however, showed that *Paracoccidioides* reproduced in the tissues by multiple-budding, thus in-

* Aided by a grant from the John and Mary R. Markle Foundation.

† National Research Council Fellow in the Medical Sciences, Duke University School of Medicine, 1939-40.

1 Gilchrist, T. C., and Stokes, W. R., *J. Exp. Med.*, 1898, **3**, 53.

2 *a.* Moore, M., *Ann. Missouri Bot. Gard.*, 1933, **20**, 49; *b.* Moore, M., *Ann. Missouri Bot. Gard.*, 1933, **20**, 471; *c.* Moore, M., *Arch. Derm. and Syph.*, 1933, **27**, 49.

3 Castellani, A., and Jacono, I., *J. Trop. Med. and Hyg.*, 1933, **36**, 297.

4 Benham, R. W., *Arch. Derm. and Syph.*, 1934, **30**, 385.

5 Ciferri, R., and Redaelli, P., *Att. dell' Ist. Bot. Univ. di Pavia*, 1935, **6**, 55.

6 Lutz, A., *Brasil-med.*, 1908, **22**, 121, 141.

7 Fonseca, O. da, and Leao, A., *Compt. Rend. Soc. Biol.*, 1928, **98**, 619.

8 Almeida, F. de, *Ann. Fac. Med. Sao Paulo*, 1929, **4**, 91.

9 Almeida, F. de, *Compt. Rend. Soc. Biol.*, 1930, **105**, 315.

10 Moore, M., *Arch. Derm. and Syph.*, 1938, **38**, 163.

dicating its relationship to *Blastomyces*, but retained the name *Paracoccidioides* and placed it in his family Coccidioideaceae in equal rank with *Coccidioides* and *Rhinosporidium*.

Although the stages in the life cycle of *Blastomyces dermatitidis* and *Paracoccidioides brasiliensis* have been studied by numerous investigators,[1–11] and *Paracoccidioides* has been compared with *Coccidioides*,[8–10, 12] no detailed comparative study of the various forms of *B. dermatitidis* and *P. brasiliensis* has been made. In order to establish the identity of the South American fungus the present study was undertaken.

Seven strains‡ of *Blastomyces*, isolated from lesions of North American blastomycosis, and 6 strains‡ of *Paracoccidioides*, isolated from lesions of the South American disease, were compared as to their appearance in tissue, and their growth and microscopic appearance on various artificial culture media both at room and at incubator temperature. *Blastomyces dermatitidis* was seen in the tissues as single-budding, thick-walled, yeast-like organisms. When diseased tissue or pus from blastomycotic lesions was placed on Sabouraud's glucose agar, after varying periods of time at room temperature, the fungus developed into a cottony-mold-like growth. Microscopically this growth was composed of a filamentous, branching, septate mycelium with numerous spores borne sessile or on short pedicles from the hyphae. In old cultures these spores developed into large, thick-walled, variously sculptured chlamydospores. When this cottony mycelium was transplanted to blood, glycerine, Sabouraud's dextrose, beef infusion or beef extract agar and incubated at 37°C, after varying periods of time the resulting growth was smooth and waxy or cerebriform and wrinkled, and lacked the aerial, mycelial growth typical of the cultures maintained at room temperature. Microscopically this growth consisted of yeast-like, budding forms similar to those seen in diseased tissue or pus.

Paracoccidioides brasiliensis was seen in diseased tissues as multiple-budding, thick-walled, yeast-like cells. As in *Blastomyces*, when diseased tissue, obtained from experimental animals, containing this organism was placed on Sabouraud's dextrose agar, after varying periods of time at room temperature, the organism developed slowly, producing either cerebriform cultures similar to *Achorion Schoen-*

11 Martin, D. S., and Smith, D. T., *Am. Rev. Tuberc.*, 1939, **39**, 275.

12 Jordan, J. W., and Weidman, F. D., *Arch. Derm. and Syph.*, 1936, **33**, 31.

‡ The list of these strains with detailed information as to their sources will be published in a later paper.

leini or cottony cultures with well-developed aerial hyphae. Microscopically these cultures were composed of a filamentous, branching, septate mycelium with intercalary, lateral and terminal chlamydospores. When this growth was transplanted to blood, Sabouraud's dextrose, beef infusion or beef extract agar and incubated at 37°C, after varying periods of time the fungus converted to a waxy and smooth or cerebriform growth. Microscopically this growth was composed of multiple-budding, yeast-like cells, identical with the forms seen in tissue.

Since these 2 fungi behave in a similar manner in tissues, in cultures at room temperature and in cultures at incubator temperature, it would seem that the differences noted above should be considered of specific rather than of generic importance. It is the writers' opinion, therefore, that only a single genus should represent the several isolations of fungi from North American blastomycosis and the several isolations of fungi from South American blastomycosis. For this reason the new combination, *Blastomyces brasiliensis,* is proposed for the fungi which produce South American blastomycosis. Although *Blastomyces* is not a suitable name for this genus because of the rules of priority in botanical nomenclature, it should be retained for these fungi until some generally accepted name is agreed upon. As only one species causes North American blastomycosis and the various species reported from South American blastomycosis[10, 18] differ only slightly in cultural aspects and morphology, only 2 species need to be considered, namely, *Blastomyces dermatitidis* Gilchrist and Stokes 1898 and *Blastomyces brasiliensis* (Splendore) Conant and Howell, comb. nov.

12016 P

Effect of Injections of Nuclei on "Take" of Implants of a Lymphoma in Mice.

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From the Crocker Radiation Laboratory, University of California, Berkeley.

In these experiments we have attempted to determine whether or not, by the injection of nuclei of liver and of tumor cells, it is pos-

[13] Moore, M., *Rev. biol. hyg.*, 1935, **6**, 148.

* Finney-Howell Research Fellow.

† Wm. R. Kenan, Jr., Fellow.

sible to immunize mice against a lymphoma which, on implantation, invariably grows till the host is killed. Stoneberg and Haven[1] have reported inhibition of growth of the Walker carcinoma 256 after injection of serum of rabbits which had received injections of nuclei of the tumor. Since spontaneous regression and failure of transplants to "take" occurs rather commonly when this tumor is used, it seemed doubtful that definite conclusions could be drawn from their experiment.

Materials and Methods. The Gardner-Lawrence[2] lymphoma used in these experiments has been transplanted into about 40 generations of the highly inbred Strong "A" strain of mice.

Four types of suspensions were injected subcutaneously: (1) Approximately 500 viable lymphoma cells; (2) fragmented lymphoma cells prepared by successively freezing in liquid air, grinding and thawing 3 times; (3) nuclei of lymphoma cells; and (4) nuclei of livers of A strain mice. The method of isolating nuclei has been described.[3] The material of (1) and (2) was suspended in 0.9% saline. The nuclei of the first 8 injections of (3) and the first 5 injections of (4) were suspended in 5% citric acid. Since this produced cutaneous ulceration, the last 6 injections of nuclei were suspended in isotonic McIlvaine buffer at pH 7.0 which produced no local reaction.

Fragments of the lymphoma were implanted subcutaneously into 400 control A strain mice and in no case did the tumor fail to grow; 2500 A strain mice received intraperitoneal injections of 15,000,000 lymphoma cells and in all cases a lymphoma developed and grew. No regression of this tumor has ever been observed.

Results and Conclusions. In Table I are summarized the number and type of "immunizing" injections administered and the number of survivors of 3 subsequent tumor implants given 1 month apart. The "immunizing" treatment in (1) and (2) killed over half of the animals, most dying of lymphoma, some of toxemia. The injections in (3) and (4) resulted in a mortality rate little more than would be expected in an untreated group in 4 months (9.5%). None developed tumors following injections of nuclei.

Of the 100 mice receiving a single injection of 500 viable lymphoma cells only 2 survived. Of these, one survived 2 implantations before succumbing to pneumonia and the other survived 3 implantations and at the time of this writing is still alive without tumor. Since

1 Stoneberg, C. A., and Haven, F. L., *Am. J. Cancer,* 1940, **38**, 377.

2 Lawrence, J. H., and Gardner, W. U., *Am. J. Cancer,* 1938, **33**, 112.

3 Marshak, A., *Science,* 1940, **92**, 460.

TABLE I.

	1 Viable lymphoma cells to 100 mice	2 Fragmented lymphoma cells to 90 mice	3 Lymphoma nuclei to 95 mice	4 Liver nuclei to 90 mice
No. injections 5/15/40-9/15/40 Amt. injected per mouse	1 500 cells	10 50,000 cells	14 .003-.077 cc nuclei	11 .001-.01 cc nuclei
No. of survivors of "immunization" treatment	2	43	83	84
No. of survivors of 3 successive implants following "immunization"	1	4	2	4

the number of survivors is so small no deductions can be drawn regarding the efficacy of this treatment as means for "immunizing" the animals to the tumor. In group (2) receiving fragmented cells, 4 of the 43 survivors (9.3%) showed "immunity" to 3 successive implantations. Group (3) receiving tumor nuclei showed 2.4% "immunity" while in group (4) receiving liver nuclei there was 4.8% immunity.

Since no failures to "take" were observed in 2900 control animals inoculated with the tumor, the incidence of immune animals in this strain must be less than .035%. As a result of the treatments described, the incidence of immunity has been raised to 2.4-9.3% (which seems to be a significant increase). The injections of nuclei produce about as much "immunity" in this population of mice as the other two methods used (injection of viable and of ground frozen cells), without the risk of killing a large proportion of the animals entailed in these latter procedures. Furthermore the "immunization" obtained in treatments (3) and (4) must be attributed to a response to nuclear material only. This suggests that the "immunization" obtained by injection of various types of whole cells and macerated tissues[4] may be through response to the nuclei they contain.

[4] MacDowell, E. C., *et al.*, PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 84; MacDowell, E. C., *et al.*, *Proc. Nat. Acad. Sci.*, 1935, **21**, 507; Rhoads, C. P., and Miller, D. K., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 817.

12017

Attempted Adaptation of the Virus of Poliomyelitis to Wild Rodents.

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Since the publication of the report by Armstrong[1] that the Lansing strain of poliomyelitis could be transmitted to the eastern cotton rat, *Sigmodon hispidus hispidus*, studies were undertaken to repeat his findings using other strains of poliomyelitic virus, both recently isolated and those with long passage through monkeys. Toomey and Takacs[2] had obtained negative results using 9 strains of virus, while Kessel and Stimpert[3] reported unfavorably on the use of several wild species of mice and rats. Jungeblut and Sanders,[4] however, obtained what they called a murine strain of virus after passage of the S.K. poliomyelitic virus to cotton rats. Hammon[5] reported on negative findings using the Duran-Reynals factor to accelerate the action of the virus in rats. But Toomey and Takacs[6] have recently been able to obtain passage of the MV strain of poliomyelitis to the cotton rat after addition of their colon-typhoid-paratyphoid broth filtrate. Typical poliomyelitis could be produced in monkeys after the 6th and 8th generations of the virus in rats.

Several species of wild rodents, *Sigmodon hispidus eremicus, S. h. texianus, Microtus californicus* and *M. montanus* were obtained from the field and later raised by Dr. F. Evans of the Hooper Foundation. All but one species, *S. texianus*, were indigenous to California.

Large animals of unknown age brought in from the field were used for the first passage experiments. They were given 0.03 to 0.05 cc intracerebrally, 0.5 cc intraperitoneally and about 0.2 cc intranasally of a 10% suspension of 6 strains of poliomyelitis; Jackson, isolated in California in 1934; Reichle from Switzerland in 1934; Geise and Dyer in 1939 and Caldwell in 1940 from California. The doses mentioned were employed throughout all the succeeding inoculations unless otherwise recorded. The 13 *Microtus californicus*

* Aided by a grant from the National Foundation for Infantile Paralysis.

1 Armstrong, C., *U. S. Pub. Health Rep.*, 1939, **54**, 1719.

2 Toomey, J., and Takacs, W. S., Proc. Soc. Exp. Biol. and Med., 1940, **43**, 536.

3 Kessel, J., and Stimpert, F. D., Proc. Soc. Exp. Biol. and Med., 1940, **45**, 665.

4 Jungeblut, C., and Sanders, M., *J. Exp. Med.*, 1940, **72**, 407.

5 Hammon, W. McD., Proc. Soc. Exp. Biol. and Med., 1940, **45**, 124.

6 Toomey, J., and Takacs, W., Proc. Soc. Exp. Biol. and Med., 1940, **45**, 364.

and the 13 *Sigmodon h. eremicus* inoculated failed to show any symptoms of disease after 4 weeks' observation. Brain material from 3 other cases suspected of having poliomyelitis was also used but both the monkeys and the rodents proved negative.

Younger animals varying in age from 2 to 3 weeks were then employed. Eight *Microtus montanus*, 3 *M. californicus*, 52 *Sigmodon h. eremicus* and 7 *S. h. texianus* were inoculated, not only with the poliomyelitis strains just enumerated but with the MV strain and fresh brain material from 4 new cases, including 2 in California and 2 in Tacoma, Washington. No symptoms of poliomyelitis were noticed in any of the animals after 4 to 6 weeks' observation.

A group of wild mice of the 2 species were inoculated with the Jackson strain of poliomyelitis in the amounts previously mentioned and from 2 to 3 of these were killed at intervals of 1, 2, 3, and 4 days, and 1, 2, and 3 weeks, respectively. Each lot was then passed into 2 to 3 other normal wild mice and also to a monkey. The second generation mice coming from the original groups killed on the first, fourth, and seventh days were killed and passed to a third generation. A few of the mice died of an intercurrent bacterial infection in 2 to 3 days after inoculation, but otherwise all the others survived without any typical symptoms suggestive of poliomyelitis. On the other hand, the monkeys given the mouse brains removed on the third and fourth days after injection, respectively, both developed temperatures and typical symptoms of poliomyelitis. The wild mice inoculated with the same material, however, failed to carry on the virus.

A similar experiment was undertaken by inoculating 15 Sigmodons, 13 to 23 days old, with the Jackson strain of virus. Three to 4 animals were killed in 3 days, in 2, 3, and 4 weeks, respectively after the inoculation and their brains were passed on into 4 more cotton rats. Brain suspensions from all the rats, except those killed on the 4th week were also inoculated into monkeys but without positive results.

The Sigmodons killed on the fourth day were passed to 3 others, one of which developed a paralysis of the hindlegs on the third day. It was killed and its brain transferred to 4 other rats and a monkey. They all remained well. In 2 weeks the rats were killed and passed to a fourth generation of animals. In 2 weeks 3 of these developed a very spastic type of convulsion, characterized by a weakness and slight transient paralysis of the hindlegs, combined with rough hair, general nervousness and hyperexcitability and inclination to show a humped back. The animals usually recovered in a few minutes but were weaker for a short period. One rat died. The 3 showing

spasms were killed and passed into a fifth generation of rats. After a month one showed the same symptoms and was killed. The others remained well, as did the rats of the 6th generation given the brain from the sick animal.

Two other series of experiments were undertaken using both species of Sigmodons, 12 to 17 days of age. One group was given the Jackson strain of virus and the other the Reichle. Part of each group was killed in 4 days after the inoculation and part in 7 days and passed on both to monkeys and to other cotton rats. The former all remained well. The Sigmodons killed on the fourth day were passed through 5 generations for the Jackson strain and 4 for the Reichle, each lot of rats being killed every 4 days and then passed to the next. The latter were killed in 4 days and so on. Monkeys were also inoculated intracerebrally at the same time after each passage but none developed poliomyelitis. On the fourth passage with the rats given the Jackson strain and on the third with those given the Reichle, animals began to show the same type of transient, spastic semi-paralysis as previously described. These animals were killed and their brains transferred to 4 or 5 rats. One of 7 developed slight spasticity in the Jackson group and one of 5 in the other.

Because this peculiar type of spasticity greatly resembled the symptoms noticed for animals injected with the virus of lymphocytic choriomeningitis, it was thought that this strain might have been activated among the wild rodents. A similar type of experiment was then undertaken by passing on the brains of normal rats *(Sigmodon h. eremicus)* into others, killing them every 4 days as in the previous groups. Animals, raised in the laboratory, from 17 to 20 days old, were used. Four or 5 were inoculated each time with the same doses as for the other experiments and their brains passed to 4 other normal animals, which were killed in 4 days and so on through 7 generations. The rats all remained quite normal through the sixth passage.

Of the 5 inoculated on the seventh generation, 3 remained well, but 2 developed the same spasms as before, on the seventh day after inoculation. These were killed and passed to 3 others, one of which remained normal but 2 developed spasms on the 25th day, the eighth generation. Upon the ninth passage only one out of 7 rats showed the symptoms. No further spasticity was noticed after the eleventh generation.

Those animals that had remained well and had not been killed out of the different groups were then tested for immunity to the virus of lymphocytic choriomeningitis (l.c.m.) by intracerebral

inoculation. All of those tested became ill with typical symptoms for this virus or else died, discouraging the idea that this virus had been present among these animals. Sections of the brains from a number of the rats that had shown atypical spasms were examined by Dr. W. P. Covell of the Hooper Foundation but they failed to reveal any lesions significantly suggestive of the l.c.m. virus.

In examining the data on these transfers with the cotton rat brains, it seemed that while animals developed neurological symptoms suggestive of a virus disease, yet there was no definite evidence for the presence of a virus among the rats. With the exception of the 2 animals given the Microtus material, no monkeys and no white mice tested with any of the brains from the rats having spasms ever developed symptoms indicative of the presence of a neurotropic disease. It was, therefore, decided that the spasms were probably of a dietary nature.

It was then found that the animals that were inoculated and kept in the laboratory under observation for a long period had only been receiving sunflower seeds. They had formerly been given a more adequate diet but during the summer because of an unusual amount of work the caretakers had put in the easiest food at hand. After addition of carrots and alfalfa it was noticed that the spasms ceased among the rats. None had been recorded for the wild mice, but they had always been given oats and bread. However, the surviving rats had grown older and the convulsions had been mainly among younger animals. This would conform to a recent report by Wolbach and Bessey that a vitamin A deficiency in young, one- to two-months-old white rats produces "an overgrowth of the central nervous system in relation to its boney enclosure, resulting in mechanical damage to brain, spinal cord and nerve roots."[7] Neurological spasms might result. Such symptoms were not noticed as much in the older animals on the inadequate diet. Addition of carrots prevented the spasms.

In the present study, since the cotton rats showing these symptoms were mainly young animals and since the return to a carrot diet stopped the convulsions, it would appear that the latter were probably due to these dietary factors rather than to a virus disease. Further evidence was obtained by keeping one group of normal rats on the inadequate diet for 6 weeks, in which time spasms were developed, and then returning them to the carrot ration, when none appeared. Another group on carrots alone for the same period never developed any spasms.

[7] Wolbach, S. B., and Bessey, O., *Science*, 1940, **92**, 483.

Another observation brought out in this study referred to the susceptibility of these wild rodents to the virus of lymphocytic choriomeningitis. Because it was thought that the convulsions might be due to this virus, a large number of animals were tested for susceptibility. 0.03 cc of a 10% suspension of the W.E. strain, kindly sent by the laboratory of Dr. T. Rivers of the Rockefeller Institute, was inoculated intracerebrally into old adults (over 6 months of age), immature animals (2-6 months), and very young animals (1-2 months) of both species of Microtus and Sigmodons. It was interesting that all of the 23 *Microtus californicus* in the 3 age groups survived the inoculations, while of the 50 *M. montanus*, all survived except 54.8% of the immature group. All of the 9 adults and the 10 young remained well.

There was less resistance among the Sigmodons. 91.1% of the 35 young *S. h. eremicus* died, as did 68.4% of the 38 immature and 50% of the adults. Among the *S. h. texianus* group the resistance was higher, as 91.7% of the young and 46.7% of the immature animals survived.

It may be seen, therefore, that the *Microtus californicus* were very highly resistant to this virus, the *M. montanus* less so, while among the rats, the *Sigmodon h. texianus* were more resistant than the other species which showed a highly susceptible younger group.

Summary. Attempts to transmit the virus of poliomyelitis, both old and newly isolated strains, to the cotton rats, *Sigmodon hispidus eremicus* and *S. h. texianus* and the mice, *Microtus californicus* and *M. montanus*, were unsuccessful by the methods employed. The virus, however, could survive in the brain of the Microtus for 3 to 4 days and be transferred to monkeys but not mice. It was found that the convulsive spasms that developed among both inoculated and the normal rats were probably due to a deficient diet and not to a virus disease. *Microtus californicus* is highly resistant to intracerebral inoculation of the virus of lymphocytic choriomeningitis, *M. montanus* less so, while the Sigmodon species are more susceptible.

12018 P

Mode of Action of "Ribonuclease."

JOHN J. EILER AND FRANK WORTHINGTON ALLEN. (Introduced by C. L. A. Schmidt.)

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A thermostable enzyme, first noted by Jones[1] to be present in the pancreas, has received recent attention at the hands of Dubos and Thompson,[2] Schmidt and Levene,[3] Kunitz,[4] and Allen and Eiler.[5] Dubos and Thompson found the enzyme to effect the decomposition of ribonucleic acid from yeast. The enzyme was noted to be without action on the following substances: desoxyribonucleic acid from thymus, egg albumin, hemoglobin, Witte's peptone, a number of plant, animal, and bacterial polysaccharides, ethyl acetate, tributyrin, and an ether-soluble fraction extracted from pneumococci.

The enzyme was named "ribonuclease" and claimed not to be a phosphatase. Schmidt and Levene describe the action of the enzyme to be that of a depolymerase, and consider the name "ribonucleodepolymerase" to offer a more appropriate description of the mode of action. Kunitz has isolated the enzyme in the crystalline state, and claims that its mode of action appears to correspond to the nuclease activity described by Dubos and Thompson. Allen and Eiler have shown that the enzyme effects the liberation of an acidic group of ribonucleic acid. Titration data place the liberated acidic group in the range of a secondary phosphoric acid dissociation. An examination of the structures of the known components of the ribonucleic acid molecule shows that the secondary hydroxyl of the phosphate group conceivably may be linked with any of the following reactive groups: (a) the hydroxyl group of guanine or uracil; (b) the amino group of guanine, adenine, or cytosine; (c) the hydroxyl group of position 5 or of position 2 of the ribose; (d) the hydroxyl of another phosphate group. This last possibility, seemingly, is ruled out by the data of Allen and Eiler. However, the indications are that certain characteristics of the action of the enzyme are those of a phosphatase.

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1 Jones, W., *Am. J. Physiol.*, 1920, **52**, 203.

2 Dubos, R. J., and Thompson, R. H. S., *J. Biol. Chem.*, 1938, **124**, 501.

3 Schmidt, G., and Levene, P. A., *J. Biol. Chem.*, 1938, **126**, 423.

4 Kunitz, M., *Science*, 1939, **90**, 112; *J. Gen. Physiol.*, 1940, **24**, 15.

5 Allen, F. W., and Eiler, J. J., *J. Biol. Chem.*, 1941, **137**, 757.

In an effort to find the exact linkage hydrolyzed by the enzyme, substances that are known to contain the foregoing phosphate-hydroxyl or phosphate-amino linkages were synthesized and subjected to the action of the enzyme. The studies were conducted in the same manner as those in which the action on ribonucleic acid was noted.[5] Sodium β-glycerophosphate, disodium phenyl phosphate, sodium phenyl anilino phosphate, sodium phenyl amido phosphate, potassium diphenyl phosphate, and phenyl phosphoryl guanine were found to be unattacked by the enzyme. Thus, it is clear that the enzyme is not to be classified as any of the non-specific phosphatases, *i. e.*, phosphomonoesterase, phosphodiesterase, or phosphoamidase.

Evidence for positive enzymic action on allonucleic acid, isolated from pancreas, and upon the Hammarsten nucleoprotein of the pancreas was noted. In both of these cases, the liberation of a group characteristic of a secondary phosphate dissociation is obtained. It appears from these data that the action of the enzyme is that of a specific phosphatase. The specificity is satisfied by two conditions that are met in the molecules of ribonucleic and allonucleic acids: (a) the specific arrangement of the mononucleotides in the nucleic acid structures, and (b) the existence of a certain as yet unidentified phosphate-hydroxyl or phosphate-amino linkage.

The nucleoprotein of Hammarsten cannot be said definitely to be an homogeneous substance. It cannot be stated at this time whether or not the action of the enzyme is to hydrolyze the linkage between nucleic acid and protein or one of the components of the nucleic acid while the latter is still combined as nucleoprotein.

12019

Influence of Local Applications of Turpentine on Mammary Gland Growth and Involution.*†

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Evidence has been presented indicating that the anterior pituitary of pregnant cattle contains a factor which will stimulate the complete

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† Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 727.

growth of the alveolar system of the mammary glands of castrate virgin female mice.[1] Whether this same factor is involved in the maintenance of the gland during the lactation period is an open question. Recently, Hooker and Williams[2] reported that the application of spirits of turpentine to the nipples and surrounding skin caused a retardation in the rate of involution of the mammary alveolar system in mice from which the young had been removed. These observations suggested that turpentine might also stimulate the growth of the mammary alveolar system as well.

The object of this paper is to present evidence in confirmation of the work of Hooker and Williams and to report the failure of turpentine to stimulate the growth of the alveolar system of the mammary glands of normal and ovariectomized female mice by similar applications.

Lactating female mice: These animals were allowed to suckle their young for four days after parturition. The young were then removed, and the lactating mothers were divided into 4 groups, 2 of which received applications of turpentine to the teats and surrounding skin twice daily.

In all of the lactating female groups some involution of the mammary glands had taken place as compared with mammary glands of mice which had suckled their young for 11 days. Group II, turpentine treated, had a maintenance of the lobule-alveolar system which was superior to that of Group I, untreated. The alveoli were better preserved, 8 of the 12 animals showing evidence of secretion. Only 2 animals of the 8 in Group I showed any evidence of secretion. In Groups III and IV the process of involution had proceeded much further than that in either Group I or II. The alveolar development of the treated group was only slightly superior to that of the untreated group and that not significantly so. Thus as time proceeded the turpentine treatment became less effective in inhibiting the normal progress of involution.

If the action of turpentine in maintaining the alveolar system was

TABLE I.

Group	No. of animals	Duration of observation (days)	Treatment	Results
I	8	7	None	Involution advanced
II	12	7	Turpentine twice daily	Alveolar system well maintained
III	4	14	None	Involution far advanced
IV	4	14	Turpentine twice daily	Involution quite similar to III

[1] Mixner, J. P., Lewis, A. A., and Turner, C. W., *Endocrinology*, 1940, **27**, 888.
[2] Hooker, C. W., and Williams, W. L., *Yale J. Biol. and Med.*, 1940, **12**, 559.

mediated by a stimulus of the lobule-alveolar mammogenic hormone, it would be expected that the application of turpentine to the teats of normal and castrate virgin female mice would stimulate the growth of the alveoli of such animals.

Normal and ovariectomized virgin female mice: Twelve virgin females weighing from 12-20 g were treated twice daily with turpentine for periods varying from 4 to 8 days. In no case was any sign of lobule-alveolar development observed. This treatment was ineffective in stimulating pseudo-pregnancy.

Similar negative results were obtained with a group of 28 ovariectomized mice which received treatment twice daily for periods varying from 4 to 10 days.

Discussion. It has been shown[3] that the stimulus of nursing causes a release of the lactogenic hormone by the AP. Whether the same stimulus also influences the maintenance of the lobule-alveolar system (which rapidly degenerates after the cessation of nursing) is difficult to determine. We have been able to confirm the interesting observation of Hooker and Williams concerning the influence of local application of turpentine upon the involution process in the mammary gland. Our observations with castrate and normal virgin mice are interpreted to indicate that turpentine does not retard alveolar involution by stimulating the secretion of mammogen in the AP. Neither does turpentine applied to the teats and adjacent skin produce pseudo-pregnancy in normal females.

It is believed that the application of turpentine to the nipples and skin causes a reduction in the rate of involution of the gland through its effect of producing a local hyperemia rather than any effect through the central nervous system. By maintaining a large blood supply around the glands a condition more nearly comparable to that present during active lactation is maintained. By care in preventing the spread of turpentine from one row of glands to the other side, it is possible to show a great difference in the hyperemia of the two rows of glands. Experiments are now in progress to determine if a differential rate of involution can be obtained under such conditions.

Summary. The application of spirits of turpentine for 7 days to the nipples and adjoining skin of lactating mice weaned on the 4th day after parturition was shown to retard the rate of involution of the mammary lobule-alveolar systems. Similar applications of turpentine to castrate and normal females failed to stimulate the growth of alveoli. Pseudo-pregnancy was not stimulated in the

[3] Reece, R. P., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bul.* 266, 1937.

normal female. It is suggested that the retardation in the involution process is due to the great subcutaneous hyperemia produced by the turpentine applications.

12020

Qualitative Progesterone Assay of Pregnant Cattle AP and Extracts Having Mammary Growth Activity.*†

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It was shown in this laboratory[1, 2] that the duct system of the mammary gland could be experimentally stimulated in castrate male or female animals by the injection of estrogen while a simultaneous injection of estrogen and progestin was necessary to secure complete mammary gland growth. Since this growth could not be secured in hypophysectomized animals, it was suggested that the ovarian hormones might produce their action by stimulating the secretion of mammogenic hormones in the anterior pituitary.[3] It has since been shown that lipid extracts[4] of the AP will stimulate the growth of the mammary duct system and further, that fresh pregnant cattle pituitaries will stimulate the growth of the lobule-alveolar system.[5]

Since Gardner and Hill[6] have shown that progesterone alone will stimulate the growth of the duct system, it seemed desirable to determine whether progesterone was present in the lipid AP extracts of the pituitary in which the mammogen duct factor is present as well as in the fresh pregnant cattle AP which stimulates the growth of the lobule-alveolar system.

In previous studies of this question Corner[7] and Callow and

* Aided in part by a grant from the International Cancer Research Foundation.
† Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 728.

1 Turner, C. W., and Frank, A. H., *Mo. Agr. Exp. Sta. Res. Bul.* 145, 1930.

2 Turner, C. W., and Frank, A. H., *Science*, 1931, **73**, 295.

3 Gomez, E. T., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bul.* 259, 1937.

4 Lewis, A. A., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bul.* 310, 1939.

5 Mixner, J. P., Lewis, A. A., and Turner, C. W., *Endocrinology*, 1940, **27**, 888.

6 Gardner, W. U., and Hill, R. T., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 718.

7 Corner, G. W., *Am. J. Physiol.*, 1930, **95**, 43.

Parkes[8] reported either negative or questionable responses with pituitary extracts. The development of the more sensitive progesterone assay method of McGinty, Anderson and McCullough[9] is a further reason for a reëxamination of the possible presence of progesterone-like activity of the pituitary.

Procedure. Four immature female rabbits were sensitized over a period of 6 days with 150 i.u. of estrone. On the 7th day their abdominal cavities were opened under ether anesthesia, and the materials to be tested were injected into 3 cm isolated segments of a uterine horn. After 72 hours the isolated test segment as well as a control segment from the opposite uterine horn was removed. These were prepared for histological examination and the degree of progestational proliferation was rated according to the McPhail scale.[10]

Rabbit A received in one uterine segment 400 mg of fresh pregnant cattle AP in a water suspension. Ten out of 10 ovariectomized virgin female mice which were injected with this same material over a period of 10 days at the rate of 368 mg per mouse, responded with lobule-alveolar growth. Similarly 4 of 6 mice injected at the rate of 200 mg per mouse over a 6-day period gave positive lobule-alveolar growth.

Rabbit B received in one segment of the uterus .02 mg crystalline progesterone in .1 cc raisin seed oil. The check segment in the other horn received .1 cc oil only.

Rabbit C received in one segment .0002 mg crystalline progesterone in .1 cc oil. The control segment in the other horn received .1 cc oil only.

Rabbit D received in one uterine segment 10 mg of lipid extract No. 60 which was dissolved in .1 cc raisin seed oil. One-tenth cc of oil was placed in the control segment in the other uterine horn. This extract contained approximately 13 mouse units of the mammogenic duct growth hormone per mg.

Two virgin sexually mature rabbits (E and F) were treated as in the Clauberg test.[11] Eight preliminary daily injections, 100 i.u. each, of estrone were made subcutaneously. In the same manner lipid extracts of AP were administered daily for 5 days. Portions of uterine horns were removed and prepared for histological examination.

8 Callow, R. K., and Parkes, A. S., *J. Physiol.*, 1936, **87**, 28p.

9 McGinty, D. A., Anderson, L. P., and McCullough, N. B., *Endocrinology*, 1939, **24**, 829.

10 McPhail, M. K., *J. Physiol.*, 1934, **83**, 145.

11 Clauberg, C., *Zentralb. f. Gynäk.*, 1930, **54**, 2757.

TABLE I.
Results.

Rabbit No.	Assay method	Treatment	McPhail rating: Test segment	McPhail rating: Control segment
A	McGinty	400 mg AP	0	0 to +
B	"	.02 " Progesterone	++	0
C	"	.0002 " "	+	0
D	"	10 " Extract 60 AP	0	0
E	Clauberg	5 " " 46 "	0	
F	"	5 " " 61 "	0	

Rabbit E received 5 mg total of extract No. 46 of cattle AP prepared by vacuum distillation of 86% alcohol used in the Bergman-Turner[12] method of preparing initial extract. The residue was then fractionated in ether. Extract No. 46 constituted the ether-soluble fraction. One milligram of this extract contained approximately 10 mammogenic duct growth mouse units.

Rabbit F received a total of 5 mg of extract No. 61 containing 2 mammogenic duct growth mouse units per mg.

Extract No. 60 (pregnant cattle) and extract No. 61 (cattle pituitary) were prepared by A. J. Bergman by vacuum distillation at a low temperature (30°-35°C) of the acetone-ether used in drying the AP. Further purification was obtained by fractionation with ether. These extracts constituted the ether-soluble fractions.

Discussion. These results are believed to indicate that the mammary gland growth secured either with the fresh material or with extracts of the AP could not be due to the presence of progesterone. The amounts of progesterone administered by Gardner and Hill[6] to obtain duct growth in the male mouse varied from .35 to 1.6 Corner-Allen units (1 C-A. unit = 1 mg) over a period of 14-16 days whereas 10 mg of our extract 60 failed to give a positive progesterone response (Rabbit D).

Further, Mixner (unpublished) observed that 500 gamma of progesterone injected simultaneously with 133 i.u. of estrone over a 10-day period into castrate virgin female mice stimulated lobule-alveolar development in 1 of 4 mice, whereas 1000 gamma with the same estrone dosage caused development in 3 of 4 mice. Two-tenths gamma of progesterone gave a positive uterine response in Rabbit C. Yet a negative progesterone response was secured with 400 mg of fresh cattle AP (Rabbit A).

Summary. Fresh pregnant cattle pituitary in amounts which will stimulate growth of the lobule-alveolar system in castrate female

[12] Bergman, A. J., and Turner, C. W., *J. Biol. Chem.*, 1938, **123**, 471.

mice was found to contain insufficient progesterone to give a positive response by the sensitive McGinty technic. Lipid extracts of the AP which stimulate duct growth in the male mouse were also found to be negative for progesterone. These observations are taken to indicate that neither the mammogenic duct nor lobule-alveolar effects of the AP are due to the presence of progesterone.

12021

Effect of Selective Poisons on Utilization of Glucose by Yeast.

Morris J. Pickett and C. E. Clifton.

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Well washed suspensions of *Saccharomyces cerivisiae* (American Type Culture Collection No. 575) may utilize glucose by oxidizing it to carbon dioxide and water, by fermenting it to carbon dioxide and alcohol, and by synthesizing it to cell material. With well aerated suspensions the oxygen consumption, as determined at 37°C by the Warburg technic, is approximately one-third of the theoretical amount required for complete combustion, less than 5% of the sugar is fermented and the remainder appears to be synthesized to cellular material.

The possibility of so altering the course of glucose metabolism by yeast that the substrate will be entirely oxidized has been suggested by studies with a number of microörganisms. Clifton and Logan,[1] using resting suspensions of *Escherichia coli*, showed that glucose is oxidized to completion in the presence of sodium azide or 2:4-dinitrophenol (DNP). Similarly, Winzler[2] showed that M/1250 azide or M/2000 DNP blocks synthesis from acetate by yeast suspensions, the oxidation going to completion in the presence of these poisons.

The following results indicate that sodium azide and DNP block synthesis from glucose by yeast. However, in the presence of these poisons the oxidation does not go to completion but instead fermentation accounts for the bulk of the substrate disappearing from the medium. Typical results are reported in Table I.

In the presence of M/1000 DNP the assimilatory process is in-

1 Clifton, C. E., and Logan, W. A., *J. Bact.*, 1939, **37**, 523.

2 Winzler, R. J., *J. Cell. and Comp. Physiol.*, 1940, **15**, 343.

TABLE I.

Effect of Sodium Azide and of Dinitrophenol on Aerobic Utilization of Glucose by Yeast. Each Warburg Vessel Contained 0.1 ml of M/10 Glucose, 0.1 ml of Poison (or H_2O) and 1.8 ml of a M/15 Phosphate Buffer (pH 6.0) Suspension of Yeast.

Poison	$\mu l\ O_2$	$\mu l\ CO_2$	R.Q.	%substrate oxidized	%substrate fermented	Total
—	466	481	1.03	34.7	3.1	37.8
M/1000 DNP	495	779	1.57	36.8	63.4	100.2
M/10,000 NaN_3	543	691	1.27	40.4	30.3	70.7
M/2000 NaN_3	104	520	5.00	7.7	85.3	93.0

hibited and fermentation now accounts for two-thirds of the added substrate. The oxidative system appears to be unaffected by this concentration of DNP. Both respiration and synthesis, however, are inhibited by sodium azide, the substrate being almost entirely fermented in the presence of M/2000 azide. Although the total oxygen consumption was greater in the presence of M/10,000 NaN_3 than in its absence, the Q_{O_2} was significantly lower (Q_{O_2} normal = 90; with M/10,000 azide = 50; with M/2000 azide = 8) and there was an appreciable lag period before the rate of oxygen consumption reached a constant level. The oxygen consumption observed in the presence of azide may be attributed to oxidation of the alcohol which appears as an end product of fermentation. The rates of glucose and of alcohol oxidation in the absence of a poison are approximately equal. Oxidation of the latter compound proceeds at about 60% of the normal rate in the presence of M/10,000 azide but is almost completely inhibited by M/2,000 azide. These results suggest that fermentation may be the normal mode of glucose utilization by this strain of yeast, the alcohol produced being immediately oxidized to carbon dioxide and water. This hypothesis is difficult to reconcile with Lundsgaard's[3] report that fermentation of glucose by yeast may be blocked by monoiodoacetic acid, respiration being maintained near its normal intensity. It must be borne in mind, however, that Kluyver[4] has reported slightly higher concentrations of this poison also block respiration.

In order to confirm the manometric results a number of Warburg experiments were carried out on a semi-macro scale, subsequent analyses being made for alcohol, glycerol, glucose and reducing sugar content of the yeast cells following acid hydrolysis. The data from 2 such experiments are summarized in Table II. These results show that alcohol, only a trace of which appears as an end product

[3] Lundsgaard, E., *Bioch. Z.*, 1930, **220**, 1, 8.

[4] Kluyver, A. J., and Hoogerheide, J. C., *Proc. Akad. Wetensch. Amsterdam*, 1933, **36**, 596.

TABLE II.

Recovery of Initial and End Products from Yeast Suspension. Warburg Experiment Terminated as Soon as Rate of Metabolism Fell to Endogenous Level, and Cells Immediately Separated from Medium by Centrifugation. 10 ml Yeast Suspension in M/15 Phosphate Buffer at pH 6.0, glucose M/50, 37°C; Oxygen Atmosphere.

Medium	Glucose only	Glucose + M/10,000 NaN_3	
Mg carbon added as glucose	14.40	14.40	14.40
Mg carbon recovered as:			
CO_2	6.00	4.42	5.38
Alcohol	0.18	5.91	5.34
Glycerol	0.36	0.58	0.51
Glucose in medium	1.32	0.43	0.18
Synthesized carbohydrate	3.88	1.08	trace
Total	11.74	12.42	11.41
% carbon recovered	81	86	79

in the absence of azide, accounts for 30-40% of the added glucose when the cells are poisoned with M/10,000 azide. At the same time the synthesis of carbohydrate is markedly inhibited. A trace of glycerol is formed both in the presence and in the absence of azide.

It is also shown in Table II that, even in the presence of azide, 15-20% of the added glucose is unaccounted for as end products. A similar discrepancy was observed when the amount of synthesis was determined by increase in dry weight of the cells. Since tests for acetyl-methyl-carbinol and volatile, pyruvic, and lactic acids were consistently negative or showed only traces of these compounds, it has been tentatively assumed that there may be an accumulation of non-reducing phosphate esters in the experimental medium. Further evidence supporting this hypothesis will be reported at a later date.

Summary and Conclusions. The synthesis of carbohydrate from glucose by resting yeast is completely inhibited by M/1000 2:4-dinitrophenol. Fermentation, however, is markedly stimulated, while respiration appears to be unaffected. Both synthesis and respiration are inhibited by M/10,000 sodium azide, but a portion of the alcohol formed by fermentation of the glucose is later oxidized. Higher concentrations of azide also inhibit the oxidation of alcohol, thus permitting the accumulation of alcohol as an end product of the utilization of glucose.

12022

Failure of the Gas Gangrene Organism to Proliferate Within Nerve Bundles.

Benjamin Kropp and Maitland Baldwin. (Introduced by E. M. Boyd.)

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In the course of a study of experimental gas gangrene in guinea pigs, the relative resistance of nerves to invasion by *Cl. welchii* was frequently noted. The literature on the histopathology of gas gangrene of the peripheral nervous system gives few details of conditions within nerves. In one case of human gas gangrene from which we obtained postmortem material, however, examination of microscopic sections of the groin musculature showed a single *Cl. welchii* lying between axis cylinder and myelin sheath. This nerve was contained within an edematous nerve bundle and the latter was in the midst of a necrotic area. This one observed instance of an organism in this position so emphasized the usual absence of these organisms from very close proximity to nerve fibers in both experimental infection in guinea pigs and natural infection in man, that an attempt was made to induce growth and proliferation of the organisms by depositing them directly within the nerve bundles.

The growth* from 5 cc of the supernatant of a 24-hour chopped meat culture of *Cl. welchii* (strain WX) was suspended in 1 cc of 10% $CaCl_2$. Of this suspension, 0.1 cc was injected directly into the sciatic nerve of the guinea pig on one side in 6 animals. The needle, 27 gauge, was directed proximally. It penetrated the nerve about 2 cm distal to the sciatic notch and was pushed forward into the nerve for a distance of about 0.5 cm. The object was to deposit the organisms either within the nerve bundle or very close to a nerve bundle. This succeeded in most cases and was checked by following the rupture of epineurium and perineurium caused by the needle, and also by occasionally finding the organisms between nerve fiber and myelin sheath at the place of injection. In the latter cases, judged by their staining reaction the organisms were always dead before the nerve was fixed. There was never clear evidence of proliferation of the organisms at this site.

After 40 hours, the animals were sacrificed and the sciatic nerve

* We are greatly indebted to Dr. J. H. Orr of the Department of Bacteriology for the bacterial cultures and other assistance.

examined. Conduction distally through the nerve was almost non-existent in most cases. The nerve was then excised from the sciatic notch to a few millimeters beyond the point of insertion of the needle, fixed in 10% formalin, sectioned in paraffin and stained by Goodpasture's modification of the Gram method. The vertebral canal was opened, the spinal cord examined, and in some cases the latter was also prepared by the above method. Examination of sciatic nerve sections never revealed active organisms lying within the myelin sheaths of nerves or between sheaths. The connective tissue sheaths outside the nerve bundles, however, invariably showed large numbers of organisms in active proliferation in both proximal and distal directions. Although devoid of organisms, nerve fibers, myelin sheaths and adjacent connective tissue showed varying degrees of edema. Animals permitted to die of the infection frequently showed extreme necrosis of these tissues. Only after the nervous tissue had been reduced to a pulpy mass, the original nature of which was only just recognizable, were the organisms found invading the debris.

In one case the infection was found to have invaded the vertebral canal, but microscopic sections of the distal portion of the cord and its coverings showed the nervous tissue to be free of organisms while the meninges were freely attacked. This infection had undoubtedly travelled through the connective tissue and epineurium by way of the sciatic notch, and entered the vertebral canal through vertebral foramina. Nowhere along this pathway were the organisms detected within nerve bundles, but were found in profusion in adjacent epineurium and connective tissue. The same result was also obtained by depositing the organisms within the connective tissue around the sciatic notch.

Even in loosely organized tissue the effects of the gas gangrene toxin are usually recognizable well in advance of its actual invasion by the organisms. Apparently, the less dense the tissue or organ the more rapid is its invasion by this organism; the pronounced tendency of gas gangrene to proliferate along fascial planes is well known. It is suggested that in the case of the peripheral nerves the epineurium and surrounding areolar connective tissue offer a more favorable mechanical, and possibly physiological, base for growth and proliferation of the organism and thus successfully delay nerve invasion until the immediately available connective tissues of the region have been destroyed.

12023 P

Action of Sporogenic Aerobic Bacilli on Living and Dead Bacteria.

L. Rosenthal.

From the Laboratories of Israel Zion Hospital, Brooklyn, New York.

The antagonistic action of sporogenic aerobic bacilli against various bacteria *in vitro* was studied by Nicolle,[1] Pringsheim,[2] Much and Sartorius,[3] myself,[4] and others. Recently Dubos[5] isolated from a sporogenic aerobic soil bacillus a very potent extract capable of destroying Gram-positive bacteria but not affecting Gram-negative.

The purpose of this investigation was to compare the action of sporogenic bacilli on living with that on dead bacteria. The following technic was used.

Thirty-one strains of the *subtilis-mesentericus* group which were isolated in our laboratory at various times were tried against the following test-bacteria: 7 Gram-positive species—*Staphylococcus aureus, Sarcina flava,* air coccus, *Pneumococcus* type I, *Streptococcus hemolyticus, Streptococcus viridans, B. diphtheriae;* and 7 Gram-negative species—*B. coli, B. typhi, B. paratyhpi B, B. dysenteriae Shiga, B. prodigiosus, B. proteus* × *19, B. pyocyaneus.* The growth of 24-hour living agar culture of a test-bacterium was suspended in 5 cc of saline and one drop of the suspension was evenly spread with a swab over the surface of an agar plate. In the case of *S. hemolyticus, S. viridans* and *Pneumococcus,* the sediment of a 24-hour culture in tryptose phosphate broth (Difco) was used for inoculating the surface of a serum agar plate. Immediately thereafter, upon the surface of this implanted plate, was made a pin-point inoculation of the sporogenic strain. After 24 hours of incubation at 37°C the plates were examined. A clear transparent zone surrounding the colony of the sporogenic strain and contrasting with the good growth of the test bacteria over the remainder of the plate gave evidence of inhibition of growth. The action of sporogenic strains on dead bacteria was studied by means of plates of agar which were mixed with a thick suspension in saline of a culture of test bacteria boiled

[1] Nicolle, M., *Annales de l'Inst. Past.,* 1907, **21**, 613.

[2] Pringsheim, E. G., *Centralb. Bakt. u. Par.,* 2 *Abt.,* 1920, **51**, 72.

[3] Much, H. u., Sartorius, F., *Med. Klin.,* 1924, **20**, 347.

[4] Rosenthal, L., *Compt. Rend. Soc. biol.,* 1925, **92**, 7; *ibid.,* 472; *ibid.,* **93**, 1569; *ibid.,* 1926, **95**, 10.

[5] Dubos, R. I., *J. Exp. Med.,* 1939, **70**, 17, 256; *Ann. Int. Med.,* 1940, **13**, 2025.

for 5 minutes. On the surface of such a turbid agar plate were made pin-point inoculations of various sporogenic strains. The formation, after 24 hours of incubation, of clear transparent zones around the sporogenic colonies gave evidence of lysis, and absence of clarification of the turbid agar showed that the dead bacteria were not affected by the corresponding sporogenic strain. With living bacteria the following results were obtained: Of the 31 sporogenic strains 7 were found to be inactive both for Gram-positive and Gram-negative species. Of the remaining 24 strains, 6 were active only against some Gram-positive bacteria but failed entirely to affect the Gram-negative group, and, finally, 18 affected organisms of both groups. In the Gram-positive group: *Sarcina flava* was inhibited in its growth by 24 strains, *Staphylococcus aureus* by 21 strains, Air Coccus by 21 strains, *B. diphtheriae* by 19 strains, *Pneumococcus* Type I by 11 strains, *Streptococcus hemolyticus* by 11 strains, *Streptococcus viridans* by 9 strains; in the Gram-negative group: *B. dysenteriæ Shiga* by 14 strains, *B. prodigiosus* by 14 strains, *B. typhi* by 12 strains, *B. proteus* by 11 strains, *B. coli* by 3 strains, *B. paratyphi B* by 2 strains, *B. pyocyaneus* by no strains. In experiments with dead bacteria all the 31 sporogenic strains, including the 6 which were found to be deprived of any antagonistic properties against living bacteria, produced lysis of all 7 Gram-negative organisms, but failed to affect in any visible way the bacteria of the Gram-positive group.

Conclusion. The tested living Gram-positive organisms were more susceptible to the antagonistic action of sporogenic aerobic bacilli than the living Gram-negative. In contrast, all tested dead Gram-negative bacteria were susceptible to the action of all sporogenic strains, while dead Gram-positive remained unaffected.

12024

Biotin Synthesis by Microörganisms.

MAURICE LANDY AND DOROTHY M. DICKEN. (Introduced by Paul György.)

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It has been postulated that many identified growth factors, and others still unrecognized, are required by microörganisms in general, and that many types of organisms do not require these growth factors

preformed since they presumably can synthesize them from simple nutrient materials.[1]

In order to determine whether the above postulate holds true for biotin, a study was made of the synthesis of this growth factor by microörganisms. It was necessary to select for study only organisms which could be cultured satisfactorily in completely synthetic biotin-free media. A wide variety of such organisms were studied. The media used in this study varied with the particular organism investigated. All, however, were completely synthetic in composition and biotin-free.

Inocula were kept to a minimum and cultures were incubated until maximum growth had been obtained. Cultures were hydrolyzed with strong mineral acid to release any biotin in the bacterial cells.[2] To each was added 0.5 ml of concentrated HCl and they were autoclaved at 15 pounds pressure for one hour. The cultures were neutralized and assayed for biotin by the yeast growth method of Snell, Eakin and Williams.[2] This method has in our hands shown a consistent sensitivity to quantities of biotin as small as 0.1 millimicrogram. The reference standard employed in all assays was a sample of crystalline biotin methyl ester, supplied by Dr. Vincent du Vigneaud. Culture medium and toxic inhibition controls were run with all assays.

As will be noted in the examination of Tables I and II, biotin is apparently not required preformed for the organisms investigated, all of which grew in synthetic media and elaborated biotin to a greater or lesser degree. In addition it will be noted from Table II that by far the largest quantities of biotin produced was found in the cell-free medium. It was not possible to definitely ascertain whether this was due to autolysis of the bacterial cells or whether the biotin was regularly secreted into the medium. The *Azotobacter* cultures were incubated for 5 days, the others for a period of 3 days.

It is necessary to emphasize that these results are only indicative and are not to be regarded as absolute for the various organisms reported. Several variables such as culture strain, medium, inoculum, incubation time and hydrolysis or autolysis may all affect the final biotin concentration.

It would be highly improbable that the organisms tested, all of different types and all chosen haphazardly, should in every instance synthesize a substance which we at present know to be required by

[1] Koser, S. A., and Saunders, F., *Bact. Rev.*, 1938, **2**, 145.

[2] Snell, E. E., Eakin, R. E., and Williams, R. J., *J. Am. Chem. Soc.*, 1940, **62**, 175.

TABLE I.
Biotin Synthesis by Microörganisms Grown in Amino Acid—Glucose Medium.

Culture	mγ Biotin per 9 cc culture*
Mycobacterium tuberculosis	215
Proteus vulgaris	89
Alkaligenes fecalis	16
Escherichia coli	273
Bacillus anthracis	20
Aerobacter aerogenes	144
Serratia marcescens	126
Eberthella typhi	226
Pseudomonas aeruginosa	46
Staphylococcus aureus	226
Sarcina lutea	14
Klebsiella pneumoniæ	72
Bacillus subtilis	44
Monilia albicans	3
Penicillium	9
Epiphyton interdigitale	5
Mucor	5
Aspergillus niger	14
Aspergillus oryzæ	11

mγ = millimicrogram or 0.001 μg.
*Cultures hydrolyzed by autoclaving with strong acid to yield maximum values.

TABLE II.
Biotin Synthesis by Bacteria Grown in Synthetic Media in 1 Liter Volumes: Distribution of Biotin in Cells and Culture Medium.

Culture	mγ Biotin per 1000 cc culture			
	Medium	%	Cells*	%
Klebsiella pneumoniæ	12,540	97.1	375	2.9
Escherichia coli	950	82.9	163	17.1
Aerobacter aerogenes	3,382	90.1	336	9.9
Alkaligenes fecalis	338	84.0	53	16.0
Bacillus subtilis	380	96.9	12	3.1
Azotobacter vinelandii (W)	19,380	95.5	874	4.5
Azotobacter vinelandii (B-1)	15,200	88.6	1786	11.4

*Acid hydrolyzed to release bound biotin.

only a few bacterial species. Biotin is probably synthesized by many other organisms which can grow in synthetic media. Our evidence indirectly supports the postulate that biotin may be required as a growth essential by bacteria besides the *Clostridia*,[3, 4] *Rhizobia*,[5, 6] and *Staphylococci*.[7, 8]

[3] Snell, E. E., and Williams, R. J., *J. Am. Chem. Soc.*, 1939, **61**, 3594.

[4] Peterson, W. H., McDaniel, L. E., and McCoy, E., *J. Biol. Chem.*, 1940, **133**, lxxv.

[5] Nilsson, R., Bjälfve, G., and Burstrom, D., *Ann. Landw. Hochschule Schwedens*, 1939, **7**, 301.

[6] West, P. M., and Wilson, P. W., *Enzymologia*, 1940, **8**, 152.

[7] Kogl, F., and Van Wagtendonk, W. J., *Recueil des Travaux Chimiques de Pays-Bas*, 1938, **57**, 747.

[8] Porter, J. R., and Pelczar, M. J., *Science*, 1940, **91**, 576.

Summary. A wide variety of bacterial species and several molds grown in synthetic biotin-free media have been shown to synthesize biotin to a greater or lesser degree as measured by the yeast-growth biotin assay method. This evidence suggests that biotin may be of widespread importance in microbial nutrition.

12025 P

Inactivation of Testosterone Propionate by Normal Female Rats.

GERSON R. BISKIND. (Introduced by Charles L. Connor.)

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The presence of androgens in normal female animals predicates a system for their inactivation and removal. The origin of these androgens is considered to be in either the ovary[1] or the adrenal.[2] The following experiment shows that one site of inactivation for these substances is the liver, just as it is for estrogens[3, 4] and androgens[3, 5] in female and male castrate rats respectively.

Pellets of testosterone propionate* produce anestrus in adult female rats when implanted in the subcutaneous tissues.[6] The duration of production of anestrus, as determined by daily vaginal smears, coincides with the presence of the pellet in the tissues. Anestrus results from inhibition of the hypophysis leading to suppression of the gonadotropic factor.[2] After the pellets are removed estrus cycles resume their normal pattern; however, occasional periods of estrus may predominate. This effect of the pellets of testosterone propionate on the estrous cycle was tested in 5 normal adult female rats for a period of 60 days. The average absorption from the pellets was .050 mg per day.

A pellet of testosterone propionate implanted in the spleen of an adult normal female rat causes no significant change in the cyclic

1 Hill, R. T., *Endocrinology*, 1937, **21**, 495.

2 Koch, F. C., *Physiol. Rev.*, 1937, **17**, 153.

3 Biskind, G. R., and Mark, J., *Bull. Johns Hopkins Hosp.*, 1939, **65**, 212.

4 Biskind, G. R., *Endocrinology*, in press.

5 Biskind, G. R., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 259.

* Supplied as Perandren through the courtesy of the Ciba Pharmaceutical Products, Inc., Summit, N.J.

6 Mark, J., and Biskind, G. R., *J. Clin. Endocrinol.*, in press.

appearance of estrus, except for slight dominance of the cornified phase in some animals. This procedure was observed in 10 normal adult female rats for a period of 60 days, by means of daily vaginal smears. The average absorption from each pellet of testosterone propionate was .072 mg per day.

Collateral anastomotic circulation from the spleen into the systemic circulation may be established by transplanting it between the overlying muscles and skin. Return of any blood into the portal system may then be prevented by ligation of the splenic vessels. A pellet of testosterone propionate implanted in such a spleen produces anestrus exactly comparable to that produced by the pellet in the subcutaneous tissues. This effect was observed in 4 normal adult female rats for a period of 30 days, by means of daily vaginal smears. The average absorption from each pellet was .081 mg per day.

Summary. Anestrus occurred as long as a pellet of testosterone propionate was present in the subcutaneous tissues of normal female rats. No significant changes occurred in the estrous cycles of normal adult female rats when pellets of testosterone propionate were implanted in the spleen in which the normal portal circulation was intact. When the blood from the spleen containing a pellet of testosterone propionate entered the systemic circulation without passing through the liver, estrus was inhibited.

The technical assistance of Ruth Helmuth is gratefully acknowledged.

12026

Rabbit Test for Thermal Reactions Caused by Concentrated Horse Serum.

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(Introduced by Ralph S. Muckenfuss.)

From the Bureau of Laboratories, New York City Department of Health.

Since tests in rabbits for pyrogenic activity of therapeutic antipneumococcic rabbit serum processed with heat and Kaolin have proven useful in indicating which preparations will produce thermal (chill) reactions when given intravenously in man,[1] the possibility of applying this method to concentrated antipneumococcic horse

1 Goodner, K., Horsfall, F. L., and Dubos, R. J., *J. Immunol.*, 1937, **33**, 279.

serum was investigated. Tests were carried out to determine whether any correlation exists between temperature response in rabbits and thermal (chill) or other untoward response in man, following intravenous administration of the same sera. Fifty-three preparations of horse serum, chosen for the most part because they were known to produce thermal or other reactions when administered intravenously in man, were studied in rabbits. No correlation was observed between temperature response in rabbits and reactions other than thermal (chill) reactions in man, and only thermal reactions are considered below.

Method. Three normal, healthy rabbits with temperatures between 99° and 102°F were each given 2 cc of one serum preparation intravenously. Rectal temperature observations were made after 30, 60, 120 and in some instances 150 minutes. The greatest elevation of temperature over the initial level was recorded for each rabbit, and the average for the 3 rabbits was computed.

The thermal (chill) effects of the serum preparations when they were used in the treatment of human pneumonia patients were compared with the average rabbit temperature response. An average temperature elevation of 1.3°F or less was found in rabbits for 14 serum lots. Nine or 64% produced no chills; 5 or 35.7% produced chills infrequently; and none caused chills frequently. Chills in less than ⅓ of patients were considered infrequent and chills in ⅓ or more were considered frequent. An average temperature elevation of 1.4°F or more was found for 39 serum preparations. Three lots of 7.7% caused no chill reactions; 12 or 30.8% caused chills infrequently; and 24 or 61.5% caused chills frequently.

It is apparent that the results in rabbits made a certain degree of selection of horse serum lots possible with regard to the tendency of the lots to cause chills when given intravenously in man. The pyrogenic activity of various sera was not associated with the degree to which the potency of the refined preparations was increased over that of the raw serum from which the lots were prepared.

Conclusions. Measurement of the pyrogenic activity of horse antipneumococcic sera in rabbits will aid in the production of concentrated serum free of chill-producing substances.

12027

Relative Metabolic Rates of Semen, Seminal Plasma, and Bacteria in Semen of the Boar.*

C. F. Winchester and Fred F. McKenzie.

From the Animal Husbandry Department, University of Missouri, the United States Department of Agriculture coöperating.

It is generally assumed that seminal plasma is an inert fluid incapable of respiration, and that the gaseous exchange of semen quantitatively represents that of the sperm.† However, in the words of Thornton and Wood,[1] "the modern conception of organic complexes in solution is that they respire, consuming oxygen." This suggested that seminal plasma may be capable of extra-cellular respiration. Seminal plasma is a relatively complex fluid as is indicated by the work of Goldblatt,[2] who has shown that seminal plasma of man contains various proteins as well as diastase and thrombokinase. The presence of catalase in semen of man was reported by Kurzrok and Miller.[3] Likewise, McKenzie, Miller, and Bauguess[4] have shown that seminal plasma of the boar contains an appreciable amount of nitrogenous material and some glucose.

Extra-cellular respiration in seminal plasma could constitute a source of error in measurements of sperm metabolism. To eliminate the possibility of an unrecognized error from this source, we have measured the relative metabolic rates of semen and seminal plasma of the boar. In addition, we have investigated the possibility that bacteria‡ may constitute a source of error in measurements of metabolism of boar's semen.

Semen was obtained from 2 normal boars, and from one double cryptorchid animal, with the artificial vagina (McKenzie[5]). Differences in concentration, motility rating, and metabolic rate of the

* Contribution from the Department of Animal Husbandry, Missouri Agricultural Experiment Station. Journal Series No. 725.

† "Sperm" is used here as a contraction of "spermatozoa."

1 Thornton, H. R., and Wood, F. W., *Canad. J. Res.*, 1935, **12**, 295.

2 Goldblatt, M. W., *Biochem. J.*, 1935, **29**, 1346.

3 Kurzrok, R., and Miller, E. G., *Am. J. Obstet. and Gynec.*, 1928, **15**, 56.

4 McKenzie, Fred F., Miller, J. C., and Bauguess, L. C., *Mo. Agr. Exp. Sta. Res. Bul.* 279, 1938.

‡ No attempt at identification of the microörganisms found in boar's semen was made. The term "bacteria" is employed here to include all of the microorganisms present.

5 McKenzie, Fred F., *The Cattleman*, Sept., 1939.

ejaculates of the 2 normal boars were not greater than the day to day variations in the semen of either animal. Sperm were separated from seminal plasma by centrifugation at gravity × 1400. Metabolism of the ejaculates was measured at 37°C with a modified Barcroft-Warburg respirometer§ (Dixon[6]).

Seminal plasma of the boar consumed oxygen in definitely measurable amounts which varied from 5 to 22% of that of the whole semen from which it was obtained. A white precipitate, which appeared when carbon dioxide-free air was passed over seminal plasma and into $Ba(OH)_2$, was considered qualitative evidence of CO_2 production. This precipitate was obtained even after the plasma had been held at 100°C for 5 minutes. The R.Q. of plasma, measured with the respirometer, was unity. Oxygen consumption of boar's semen and seminal plasma is given in Table I.

It is unlikely that the figures for oxygen consumption of plasma

TABLE I.
Relative Metabolic Rates of Semen and Seminal Plasma of the Boar, and of Seminal Plasma Before and After Treatment to Kill Microörganisms

	Semen		Seminal plasma before treatment		Seminal plasma after treatment			
No. of observations	O_2 consumption* per cc/hr, mean, mm^3	Bact.†	O_2 consumption* per cc/hr, mean, mm^3	Bact.†	Treatments used to kill microorganisms	Bact.†	O_2 consumption* per cc/hr, mean, mm^3	Change in O_2 consumption following treatment, %
1	72.7	—	8.9	—	Formaldehyde, 1	—	9.2	+ 3.4
3	126.2	+	10.0	+	drop 10% solution	—	10.7	+ 7.0
					in 3 cc plasma			
1	64.3	—	13.5	—	Merthiolate, 1 cc	—	8.1	—40.0
1	69.7	—	7.5	—	0.1% solution in	—	4.6	—38.7
5	93.5	+	10.4	+	2 cc plasma	—	6.4	—38.5
1	71.7	+	9.1	+	Mercuric chloride,	—	7.5	—17.6
1	144.3	+	7.2	+	0.1 cc 10% solution	—	6.1	—15.3
					in 1 cc plasma			
1	76.2	+	9.6	+	100°C for 5 min	—	5.2	—45.8
1	30.5	+	6.6	+		—	3.8	—42.4
3	—	—	11.5‡	—	—	—	—	—

*Volumes were measured at 37°C, and have been reduced to standard conditions of temperature and pressure.

†The + sign indicates that microörganisms were present, — that they were absent.

‡Ejaculate of a bilaterally cryptorchid boar.

§ Accuracy of the apparatus was determined by simultaneous measurements of O_2 consumption of a given sample of semen in each of the 6 manometer units. The mean deviation was less than 5%.

[6] Dixon, M., Manometric Methods, Cambridge University Press, London, 1934.

have been influenced significantly by passage of air into solution in the plasma during the measurements. In each case the materials were exposed to air for an hour, the last quarter of which was at the temperature of the experiment, before the first readings were made. Any tendency for air to dissolve in the fluids in the reaction vessels during the experiments should be balanced by a similar tendency for it to dissolve in the water in the compensation vessels. After 10 minutes in a vessel in which the pressure was reduced with a powerful filter pump, the R.Q. of seminal plasma was the same as that of a part of the sample which had not been subjected to this treatment, namely, unity.

In contrast with the sperm-free ejaculate of a human, reported by Shettles,[7] which exhibited "no measurable respiration," the sperm-free ejaculate of a double cryptorchid boar consumed about the same amount of O_2 as did normal seminal plasma (Table I).‖ That portion of the seminal plasma which passed through a porcelain filter failed to consume oxygen in each of 12 trials. This filtrate was sterile and yielded a very slight precipitate, or none at all, with sodium tungstate and H_2SO_4 (Peters and Van Slyke[8]). Thus it appears that the filtrate was free, or nearly free, of protein and of such other materials, particularly enzymes, as may have been attached to the protein molecules.

Bacteria are usually present in semen obtained from farm animals. Even though the ejaculate may be free of bacteria as it leaves the urethra, some contamination from the external genitals appears to be almost inevitable.

Measurements of errors in glycolysis determinations of ram's semen due to bacteria were made by Comstock[9] who concluded that "the effect of bacteria is negligible for at least 4 hours" as a source of error in measurements of glycolysis. We have attempted to measure errors in seminal metabolism determinations due to bacteria by comparing oxygen consumption of untreated plasma with that of plasma in which the bacteria had been destroyed. Bacteria in the plasma appeared to be approximately as numerous as those in the semen from which it was separated as judged by the number of colonies which appeared on beef-infusion medium after smears of undiluted ejaculate had been incubated for 24 hours at 37°C. Treatments

[7] Shettles, L. B., *Am. J. Physiol.*, 1940, **128**, 408.

‖ The direct method of Warburg was used in Shettles' experiments. This technic and that employed by us are considered equally accurate (see Dixon[6]).

[8] Peters, John P., and Van Slyke, Donald D., *Quantitative Clinical Chemistry*, Vol. 2, Williams and Wilkins, Baltimore, 1932, p. 682.

[9] Comstock, R. E., *J. Exp. Zool.*, 1939, **81**, 147.

used to destroy bacteria in plasma are given in the sixth column of Table I. It appears that the treatments used to destroy bacteria directly influenced the metabolism of plasma, as was indicated by the fact that the effects characteristic of given compounds appeared even when bacteria were not present in the samples, for example, those indicated in the first, third, and fourth data-lines of Table I. Since the changes in plasma metabolism following bactericidal treatments appear to be independent of effects attributable to bacteria, but characteristic of the treatments employed, it seems logical to conclude that the quantity of oxygen consumed by those bacteria present was too small to be measured by our method, and therefore completely negligible. The data given in Table I deal with the second hour after ejaculation. Some of these measurements were continued through the sixth hour after ejaculation, but the results indicate that possible errors due to bacteria were negligible even during this longer period.

Conclusions. 1. Extra-cellular respiration was exhibited by seminal plasma of the boar. Oxygen consumption of seminal plasma ranged from 5 to 22% of that of the whole semen. The R.Q. was unity. After the plasma had been held for 5 minutes at 100°C respiration at reduced intensity was observed. Respiration was not exhibited by that fraction of the seminal plasma which passed through a porcelain filter. 2. The metabolic rate of the ejaculate of a cryptorchid boar was of the same order as that of seminal plasma. 3. No errors in measurement of oxygen consumption were detected which could be attributed to the presence of bacteria.

12028

Intraperitoneal Administration of Sulfanilamide; Concentration in Peripheral Blood in Dogs.

John L. Keeley. (Introduced by Richard Ashman.)

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The reports of encouraging results following the use of sulfanilamide administered subcutaneously in the treatment of peritonitis of appendiceal origin[1, 2] have prompted the use of this drug in

[1] Ravdin, I., Rhoads, J. E., and Lockwood, J. S., *Ann. Surg.*, 1940, **111**, 53.

[2] Corry, D. C., Brewer, A. C., and Nicol, C., *Brit. M. J.*, 1939, **2**, 561.

peritonitis due to rupture or inflammation at other levels of the gastrointestinal tract.[3, 4] Sulfanilamide has also been used as a prophylactic preoperative measure in surgery of the colon and rectum.[4, 5] More recently sulfanilamide in dry form has been sprinkled about the area of peritonitis disclosed at operation for the removal of a diseased appendix or for the closure of a ruptured peptic ulcer.[6, 7] Since no report concerning the blood levels of sulfanilamide following its introduction into the normal peritoneal cavity was found in the literature, the following experiments were performed to make such determinations.

Normal healthy mongrel dogs weighing from 4.6 to 8 kg were used. There was no restriction of food, drink, or activities during the 48 hours before and after the intraperitoneal injection. After obtaining a control sample of blood from a jugular vein, a suspension of sulfanilamide crystals* in distilled water was injected into the peritoneal cavity through the upper portion of the left rectus muscle. Five animals received .3 g per kg, 2 animals received .6 g per kg, and 3 animals received 1 g per kg of the drug. Subsequent blood samples, also from the jugular vein, were obtained 15 minutes after the injection and at 2-hour intervals up to 12 hours. In some of the animals 24- and 48-hour specimens were obtained. The samples were placed in tubes containing sodium oxalate and the sulfanilamide concentration was determined by the method of Marshall.[8]

TABLE I.
Blood Concentrations of Sulfanilamide in Relation to Dosage and Intervals Following Intraperitoneal Administration.

	Dog	A	B	C	D	E	F	G	H	I	J
	Sex	F	F	F	F	F	F	F	F	F	F
	Weight, kg	6	8	6.8	5.5	4.6	6.2	6.8	5.9	5.5	6
	Dose	.3 g per kg					.6 g per kg		1 g per kg		
Blood Level of Sulfanilamide in mg% Interval after inj'n	Control	0	0	0	0	0	0	0	0	0	0
	15 min.	0.66	0.84	2.1	2.21	4.2	3.9	3.51	7.5	0.2	4.26
	2 hr	3.6	3.75	3.6	4.9	6.3	6.9	5.1	16.7	3.0	6.0
	4 "	2.7	2.49	4.5	6.6	4.6	5.7	4.5	18	5.4	7.2
	6 "	2.25	2.13	3.0	6.0	4.08	5.1	4.2	15	6.9	8.58
	8 "	1.98	1.8	—	—	—	4.2	2.85	—	7.8	6.6
	10 "	1.32	1.05	1.92	4.5	3.06	3.0	2.7	6.9	9.9	—
	12 "	1.05	0.99	1.41	3.0	2.88	1.8	2.28	6.3	4.9	6.4
	24 "	—	—	1.08	2.2	1.5	0.81	0.84	5.7	3.75	1.8
	48 "	—	—	—	—	0.16	—	—	1.11	3.0	—

3 Lockwood, J. S., and Rhoads, J. E., *S. Clin. North America*, 1939, **19**, 1457.

4 Lockwood, J. S., and Ravdin, I., *Surgery*, 1940, **8**, 43.

5 Garlock, J. H., and Seley, G. P., *Surgery*, 1939, **5**, 787.

6 O'Neil, L. J., personal communication.

7 Mueller, R. S., *J. A. M. A.* (Correspondence), 1941, **116**, 329.

* P-aminobenzenesulfonamide, manufactured by Eastman Kodak Co.

8 Marshall, E. K., Jr., *J. Biol. Chem.*, 1937, **122**, 263.

Fig 1

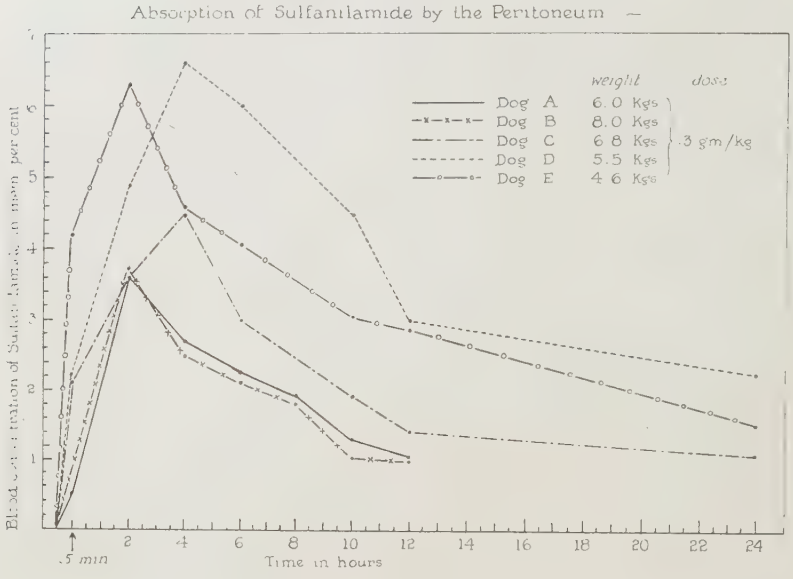
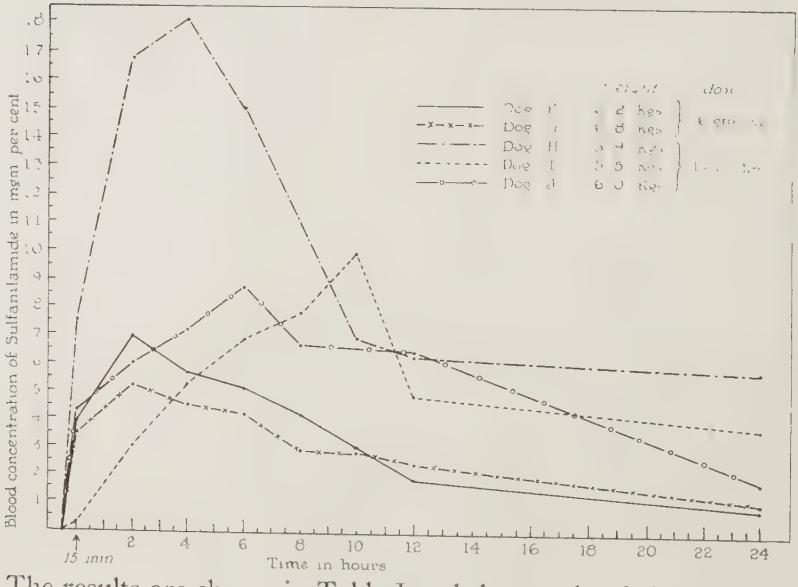


Fig 2



The results are shown in Table I and the trends of the values are presented in Figs. 1 and 2. All blood samples obtained 15 minutes after the injection contained sulfanilamide, the concentration varying

from .66 to 7.5 mg % and averaging 2.6 mg %. The higher values were associated with the larger doses.

Of the 5 animals which received .3 g per kg, the maximum concentrations of sulfanilamide were found in the 2-hour samples in 3. These peak values varied from 3.6 to 6.6 mg % and averaged 4.99 mg %. The maximum concentrations in the 2 animals which received .6 g per kg were 6.9 and 5.1 mg %. They averaged 6 mg % and were found in the 2-hour samples. In the animals which received 1 g per kg of the drug, the maximum concentrations were higher and occurred later. Thus, values of 18, 8.5 and 9.9 mg % (average 12.1 mg %) were found 4, 6, and 10 hours after injection. These maximal concentrations were followed by decreases which were less rapid in the animals receiving the larger doses and which tended to become less marked after 12 hours as judged by the 24-hour levels.

Marshall, Emerson, and Cutting[9] found that a maximum concentration of from 10 to 15 mg % was present in 3 to 4 hours after the oral or subcutaneous administration of .1 g of sulfanilamide per kg of body weight in dogs. In the present series of experiments, the maximum concentration of sulfanilamide in the blood exceeded 10 mg % in one animal only, dog "H", which received 1 g/kg of body weight. It is possible that when sulfanilamide is given intraperitoneally it is incompletely absorbed or that it may leave the blood stream rapidly, either due to absorption by the tissues or to excretion by the kidneys, thus preventing high levels of the drug in the blood. These points remain for further study.

Signs of toxicity occurred in only 2 animals, dogs "H" and "J", both of which received 1 g/kg doses of sulfanilamide. Retching, weakness, and diarrhea appeared in these animals within 2 hours. At 6 hours they had convulsions and exhibited marked extensor rigidity. Partial narcosis followed. The most severe toxic manifestations were synchronous with maximal concentrations of the drug in the blood. The animals appeared to be completely recovered in approximately 12 hours after the most severe signs of toxicity. Marshall, Cutting and Emerson[10] found no observable symptoms of toxicity with blood concentrations under 30 mg % in dogs receiving single doses of the drug orally. They point out, however, that this level of blood concentration may have been maintained for

9 Marshall, E. K., Jr., Emerson, K., and Cutting, W. C., *J. A. M. A.*, 1937, **108**, 953.

10 Marshall, E. K., Jr., Cutting, W. C., and Emerson, K., *J. A. M. A.*, 1938, **110**, 252.

only a short period of time. Concerning the relation of toxic symptoms to dosage, the same authors found that of 7 dogs receiving 1 g/kg of sulfanilamide in capsules by mouth 5 showed toxic symptoms but no mention was made of the concomitant blood levels in these animals.

Conclusions. Sulfanilamide is promptly absorbed from the peritoneal cavity in dogs. Following the intraperitoneal injection of sulfanilamide the levels of concentration of the drug in the peripheral blood are not as high as those reported by other investigators following the oral or subcutaneous administration of smaller doses.

12029

Catheterization of the Right Auricle in Man.

ANDRE COURNAND* AND HILMERT A. RANGES.* (Introduced by Homer W. Smith.)

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Forssmann[1] first used catheterization of the right heart on himself, after exposure of a vein of the arm by a surgeon. Numerous other investigators since have used right heart catheterization for visualization of the right chamber of the heart and pulmonary vascular trees by means of contrast substance.[2–7] The introduction of the Robb and Steinberg method,[8] however, renders this method unnecessary for the latter purpose. Collection of right heart blood by catheterization of the right auricle for determining cardiac output in man[9] is mentioned by Grollman,[10] who discredits it because of the possible

*Supported by a grant from the Commonwealth Fund.

1 Forssmann, W., *Klin. Wchschr.*, 1929, **8**, 2085.

2 Forssmann, W., *Muench. Med. Wchschr.*, 1931, **78**, 489.

3 Egas Moniz, Lopo de Carvalho, and Almeida Lima, *Presse med.*, 1931, **39**, 996.

4 Heuser, C., *Rev. Asoc. med. argent.*, 1932, **46**, 1119.

5 Conte, E., and Costa, A., *Radiology*, 1933, **21**, 461.

6 Ravina, A., *Progres med.*, November 3, 1934, p. 1701.

7 Ameuille, P., Ronneaux, G., Hinault, V., DeGrez, and Lemoine, J. M., *Bull. et mem. Soc. med. d. hop. de Paris*, 1936, **60**, 720.

8 Robb, G. P., and Steinberg, I., *J. Clin. Invest.*, 1938, **17**, 507.

9 Klein, O., *Muench. Med. Wchschr.*, 1930, **77**, 1311.

10 Grollman, A., *The Cardiac Output of Man in Health and Disease*, Monograph, Williams and Wilkins Co., Baltimore, 1932.

dangers and numerous misleading factors associated with it. In animal experimentation it is widely used and its innocuity established.

Because it is apparently the soundest method for obtaining mixed venous blood for respiratory gas determinations, and because of the numerous problems of hemodynamics it might help solve, a method of right heart catheterization was developed which attempts to overcome objections to former methods. The principal objections included the possibility of venous thrombi and thrombophlebitis that might be associated with introduction of a foreign body in the blood stream, the formation of thrombi within the catheter, and the psychic effects accompanying the procedure with possible alterations in the cardiac output.

The following equipment was used in our method: a specially made 10 gauge Lindeman type of needle; a 3-way stopcock with a Luer lock, tightly fitting adapter; a No. 8 French flexible radiopaque ureteral catheter with 2 holes, one at the rounded tip and another about 1 cm from the tip. The catheter is silk with a smooth varnish finish. A saline reservoir with rubber tubing and clamp for controlling the rate of flow was also used.

Under the strictest asepsis a nick is made in the skin over the median basilic vein of either the right or left arm after a preliminary infiltration with 2% novocain. After applying a tourniquet and fixing the distal portion of the vein with the fingers, the special needle is introduced. The catheter is next introduced into the vein through the needle. The end of the catheter is connected to the saline reservoir by a screw adapter, and a constant flow of saline at the rate of 15 drops per minute allowed to flow through the catheter throughout the procedure. When the catheter has been inserted about 12 cm into the vein, the needle is removed. This is done to prevent the possibility of cutting or shaving the catheter when it is withdrawn or pulled back in manipulation. The further passage of the catheter is done on a horizontal fluoroscopic table, using the fluoroscope in guiding the catheter to the desired position.

Occasionally resistance in passing the catheter may be encountered in the axillary region or at the level of the first rib. This can usually be overcome by drawing the catheter back slightly and placing the arm at a lower level than the table and rotating it inwardly. Several possible false routes may be encountered. It is conceivable that the flexible catheter might find its way into one of the jugular veins, but we have not encountered this. The catheter has on 2 occasions passed into the innominate vein of the opposite side, and occurrence immediately recognized under the fluoroscope. In either case the

TABLE I.

Influence of Right Auricle Catheterization and Arterial Blood Puncture upon Various Respiratory and Circulatory Measurements in 8 Successive Experiments.

Subject	Date	Time	Ventil. l/min	Rate per min	Gas exchange CO_2 cc/min	Gas exchange O_2 cc/min	R.Q.	Pulse per min	Venous pressure, mm	C.I.* 1/min sq.m.B.S.	S.V.* cc Beat	Observations
G.B.	10/25/40	Before	9.14	21	201	261	.77	89	132			Catheter in position 15′.
Malignant		During	9.38	22	201	275	.73	88	132†			
hypertension,	31	Before	14.57	29				93		2.29	35.5	″ ″ ″ 20′.
heart failure		During	16.42	26	221	272	.81	95		2.50	37.9	
P.T.	11/27	Before	12.67	19	223	316	.71	115		3.11	42.2	″ ″ ″ 50′.
Carcinoma		During	11.63	18	194	282	.69	114	80†	2.71	36.2	Intra-auricular pressure = 10 mm change between inspiration and expiration
liver (?)												
F.L.	12/20	Before	8.08	21	158	201	.79	79	34	2.92	61.3	Catheter in position 30′.
Carcinoma		During 1	7.78	22	168	214	.79					15′ interval between Exp. 1
stomach		″ 2	8.46	22	168	216	.79	66	34	2.72	68.3	and 2
	27	Before	7.44	19	157	200	.79	72				Catheter in position 40′.
		During 1	8.69	22	191	236	.81	66				15′ between Exp. 1 and 2.
		″ 2	9.14	25	189	237	.80	66				
	31	Before	8.32	22	167	218	.77	80		3.13	65.0	Catheter in position 25′.
		During	8.95	19	192	225	.85	68		2.80	68.4	
G.T.	1/14/41	Before	7.92	15	174	191	.91	88		2.66	45.8	Catheter in position 50′.
Carcinoma		During 1	8.72	21	178	205	.87	86				20′ between Exp. 1 and 2.
stomach		″ 2	8.07	20	158	193	.82	80		2.46	46.6	
	21	Before	6.54	16	156	208	.75	81				Catheter in position 60′.
		During 1	7.36	19	175	225	.78	80	30†	2.17	40.6	
		″ 2	7.50	19	168	221	.76	88	30†	2.29	40.8	20′ between Exp. 1 and 2.

*Stroke volume and cardiac index calculated from ballistocardiogram tracings (wave area formula) and cross-section area of the aorta (Bazett's table) using ideal weight.

†Intra-auricular pressure.

tip is withdrawn to the axillary region and further attempts made to pass it to its proper position. When the tip is in the right auricle, if blood is to be collected through the catheter, the tubing of the saline reservoir is first disconnected and a large syringe filled with a little saline is adapted to the 3-way stopcock. Two or 3 cc of blood are drawn into this syringe, thus washing the catheter with right heart blood. Then the valve is turned and the blood for analysis is collected in a second syringe containing mineral oil as an air seal. Fifteen to 20 cc of blood can be collected within 25 seconds by using only the slightest amount of suction. When the blood has been collected, the saline reservoir may be connected again and saline allowed to run slowly into the catheter to keep it open. Duplicate samples may be taken later. Finally, the catheter is withdrawn and examined carefully for any evidence of thrombi.

In our experience we have found no evidence of blood clotting on the smooth outside walls of the catheter. Nor have there been any thrombi seen at the holes of the catheter or within the catheter when it is flushed with saline after being withdrawn. We do not believe that the results are affected by any psychic disturbance. There is no pain involved in the operation, once the needle is in place in the arm vein. The pulse rate does not vary significantly before and during the procedure, and cardiac output determinations as measured by the ballistocardiograph before, during and after the procedure are quite constant.

Table I shows the influence of the catheterization associated with an arterial puncture, in 8 successive passages, upon ventilatory volume and rate, gas exchange and respiratory quotient, pulse rate and cardiac output measured with a ballistocardiograph, and in a few instances upon the venous pressure. The ballistocardiograph used differs slightly in design from that described by Starr, *et al.*,[11] and was developed in the Department of Physiology, New York University College of Medicine.

Protocol of Determination of Cardiac Output by Simultaneous Sampling of Right Auricle and Femoral Arterial Blood and Collection of Expired Air (Tissot).*

F.L. Age 61 Weight 53 kg Height 180 cm. Body surface area 1.66 sq.m.
Carcinoma of stomach.
Date—12/31/40—not basal.
Ventilation = 7.77 lit./min., dry gas 0°C, 760 mm Hg.
CO_2 Output = 2.47 %—192 cc/min.
O_2 Intake = 2.89 %—225 cc/min.
R.Q. = .854
CO_2 Content, vols.%: M.V.B.† = 54.7—art. blood = 51.2—A—V difference = 3.5.

11 Starr, I., Rawson, A. J., Schroeder, H. A., and Joseph, N. R., *Am. J. Physiol.*, 1939, **127**, 1.

O_2 Content, vol.%: M.V.B.† = 10.0 — art. blood = 14.0 — A—V difference = 4.0.

R.Q. from blood: $\frac{CO_2 \text{ A--V difference}}{O_2 \text{ A—V difference}} = .875.$

Cardiac Output, lit./min. from

CO_2 A—V difference $= \frac{192}{3.5} = 5.49.$

O_2 A—V difference $= \frac{225}{4.0} = 5.63.$

Cardiac Index = 3.35 lit./sq.m. B.S. area.
Heart rate per minute = 70.
Stroke Volume = 79.5 cc.

*Arterial blood sampling starting and ending approximately 15 seconds after mixed venous blood sampling. Total duration of sampling of both bloods 35 seconds. Expired air collected during blood sampling.

†M.V.B. (mixed venous blood) refers to blood drawn directly from the right auricle.

The protocol of one cardiac output determination by simultaneous collection of blood from the right auricle and femoral artery is included herewith. The number of simultaneous determinations of stroke volume measured by this method and compared with estimations from ballistocardiograph tracings is not large enough to warrant a statement concerning the validity of the ballistic method at this time.

12030

Middle and Old Age in Cholesterol-Fed Rats.*

RUTH OKEY.

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Most of the pathological conditions involving cholesterol deposition in tissues are characteristic of middle and old age rather than youth. Previous studies on the effect of cholesterol intake on tissue cholesterol in the rat have been made with comparatively young animals and for comparatively short periods of time.

Cholesterol-fed rats have appeared to be little the worse for their high sterol intake except in that they have developed fatty livers which have contained at least 20 times the normal percentage of

* Assistance of the Work Projects Administration in care of the animals used for this study is gratefully acknowledged. (Project 10-11578 A24.)

1 Okey, R., Gillum, H. L., and Yokela, E., *J. Biol. Chem.*, 1934, **107**, 207.

cholesterol.[1] Moreover, these rats have withstood the strain imposed by poorly balanced and vitamin deficient[2] diets only a little less successfully than their controls. Pregnancy and lactation[3] have been possible in this species and a second generation has been brought to maturity on the cholesterol-rich diet.

The present investigation was designed to show whether or not rats fed cholesterol from the time of weaning would continue to show the same powers of adaptation to the high sterol diet throughout middle and old age.

Diets and routine for care of the animals were essentially identical with those previously described.[3] Series "A" consisted of 16 male and 16 female rats fed cholesterol and a like number fed the control diet for a period of about 1½ years. Series "B" consisted of 15 control and 6 cholesterol-fed females which ate their respective diets for well over 2 years. Strictly speaking only the latter group can be considered to have attained more than middle age.

Growth in both control and cholesterol-fed rats was slightly slower than that of our stock rats, but adult size compared favorably with that of stock animals. Average weights attained by cholesterol-fed males at one year of age was 393 g, by control males, 395 g. Cholesterol-fed females at the same age averaged 261 g, controls 241 g.

Health records compared, with the exceptions noted below, quite favorably with those of stock animals.

Four female controls and 4 cholesterol-fed females were taken from series "A", bred and permitted to go through *pregnancy* and *lactation* on their respective diets with the slight modifications noted elsewhere.[3] They were then returned to the regular regime used for this experiment. They remained in excellent condition until they were killed at 450 days of age.

On the other hand, in series "A" 3 control and 4 cholesterol-fed females which had never been bred and had been kept in individual cages from the time of weaning showed evidence of severe vaginal hemorrhage when they were about 15 months of age. Autopsy of 2 animals made at the time hemorrhage was noted showed uteri much enlarged and distended with unclotted blood. In one case each horn of the uterus measured three-eighths inches in diameter. Rats which recovered spontaneously from these hemorrhages had enlarged and fibrous, and, in 2 cases, edematous uteri, but we were able to find pus in only one rat.

The 21 females of series "B", kept under as nearly the same

[2] Gillum, H. L., and Okey, R., *J. Nutr.*, 1936, **11**, 303, 309.

[3] Okey, R., Godfrey, L. S., and Gillum, F., *J. Biol. Chem.*, 1938, **124**, 489.

conditions as possible but 2 years later, added to the list only one control and one cholesterol-fed rat with uterine hemorrhage. These animals were kept until about 27 months of age. We have, therefore, no reason to assume that cholesterol in the diet was responsible for this condition. The fact that we have never observed it in stock animals is probably not significant, since females are not routinely kept in the laboratory after they cease to be useful for breeding, *i. e.*, after they are more than a year of age.

Rats of series "B" after they were 18 months or more of age, had a high incidence of tumors, some of which were apparently malignant. The small number of cholesterol-fed rats in this group makes it impossible to draw conclusions, but we may at least note that 4 out of 6 of the cholesterol-fed animals over 700 days old developed tumors of considerable size while only 3 of the 15 control animals had tumors. Two of these appeared to be subcutaneous lipomas.† Data on spontaneous occurrence of tumors in rats of this age is lacking but the incidence of tumors in our stock animals is very low.

Tissue lipids. Our technic for preparation and analyses of lipid extracts of rat tissues has been described previously.[1, 3]

The data, summarized in Table I, represent averages for animals killed while apparently healthy. It will be noted that in no tissues other than liver did the cholesterol-fed animals show more than a very slight increase in total cholesterol. Differences in free cholesterol content of tissues between cholesterol-fed and control animals were insignificant. This meant that any extra cholesterol which reached these tissues was promptly esterified. A very large proportion of the cholesterol retained in the rat's body was always to be found as liver cholesterol ester. It should be noted, however, that the actual percentage of fat and cholesterol ester in the livers of these older rats was no greater than that previously noted for younger animals on the same diet.

Discussion. Throughout these studies we were greatly impressed with the extraordinary capacity of the cholesterol-fed rats to remain in apparent good health for long periods of time. Grossly enlarged and fatty livers were present throughout the life span but histological examination of these livers showed fatty infiltration rather than degeneration of functioning tissue.

† Failure of the thermostatic control in our paraffin imbedding oven resulted in overheating and consequent loss of a large proportion of the tissues from series "B" while they were being prepared for histological examination. Consequently we are unable to report the exact nature of the tumors.

TABLE I.
Average Value for Tissue Lipids.

Series and tissue		No. rats	No. samples*	Avg age rats, days	Avg wt rats, g	Moisture, %	Fatty acid	Total Cholesterol	Free Cholesterol	Ester Cholesterol	Lecithin
							—— % of moist wt ——				
Livers											
A. CF	♂	10	10	447	369	56.8	14.8	8.7	.43	8.3	
A. N	♂	11	11	445	391	69.5	5.6	.33	.23	.10	
A. CF	♀	11	11	445	256	57.9	11.8	7.3	.33	6.9	
A. N	♀	9	9	467	273	67.8	5.9	.34	.22	.12	
B. CF	♀	3	2	830	284	60.2	10.2	8.1	.39	7.7	2.5
B. N	♀	6	1	833	303	69.2	5.5	.41	.27	.14	2.7
Lungs											
A. CF	♂	12	5	447	369	78.9	3.4	.52	.38	.14	
A. N	♂	11	4	445	391	77.8	3.8	.40	.36	.04	
A. CF	♀	11	5	445	256	78.0	3.7	.53	.40	.13	
A. N	♀	9	3	467	273	76.8	4.1	.40	.36	.04	
B. CF	♀	3	2	830	284	80.8	2.4	.67	.49	.18	2.2
B. N	♀	6	2	833	303	79.4	3.4	.51	.42	.09	1.8
Hearts											
A. CF	♂	12	5	447	369	74.3	4.3	.22	.16	.06	
A. N	♂	11	3	445	391	71.4	6.5	.17	.14	.03	
A. CF	♀	11	3	445	256	74.6	5.3	.18	.15	.03	
A. N	♀	9	3	467	273	75.6	3.6	.17	.16	.01	
B. N	♀	6	1	833	303	75.7	4.8	.22	.15	.07	1.9
Spleens											
B. CF	♀	2	1	830	284	77.0	.92	.29	.28	.01	1.3
B. N	♀	1	1	833	303	75.0	2.7	.44	.38	.06	1.7
Blood											
B. CF	♀	2	1	830	284		.285	.112	.105	.007	.283
B. N	♀	3	1	833	303		.236	.095	.059	.036	.219

CF—Cholesterol-fed animals.
N—Controls.
*Analyses were sometimes made on pooled tissue samples from several animals. Averages are weighted accordingly.

A second interesting observation was the rapidity with which cholesterol disappeared from the liver when the animals developed infections or became ill. Infections with pus-forming organisms, even when they had not progressed to the stage that the animals appeared sick, were invariably associated with lowered ester cholesterol in the liver. Four male rats with weight losses of about 50 g and lung abscesses of moderate size and apparently benign nature had total liver cholesterol values averaging 1.6%, against an average value of 8.7% for total liver cholesterol in the whole group. Two of the cholesterol-fed females which had had severe uterine hemorrhages only a week before killing had total liver cholesterol values of 0.80 and 1.6% respectively.

It is very difficult to decide whether the clearing out of stored

liver cholesterol is the result of poor absorption, of mobilization of the sterol to meet some demand imposed by the illness, or of a combination of causes. Gillum in this laboratory has shown[4] that it takes a healthy rat 6 to 9 weeks to clear out its cholesterol ester stores when changed from a cholesterol-rich to a cholesterol-poor diet. This is certainly a very much slower process than that which takes place in the sick animal. Further study of cholesterol-fed animals with infections of known type and virulence seems indicated.

Summary. Rats have been fed diets containing 1% cholesterol from weaning throughout their life span. Their growth, health and time of survival have not differed significantly from those of control animals on the same basic diet without the cholesterol.

12031

Oxidation of Some Substituted Alcohols by Rat Liver.

FREDERICK BERNHEIM AND PHILIP HANDLER.* (Introduced by W. A. Perlzweig.)

From the Department of Physiology and Pharmacology, Duke University Medical School, Durham, N.C.

The alcohol oxidase of liver can oxidize a number of lower primary alcohols.[1] The effect of substitutions in the alcohol molecule has not been studied. The following is a report on the oxidizability by rat liver suspensions of a number of such substituted alcohols.

Experimental. The liver preparation was made by chopping with scissors, grinding with sand and M/20 phosphate buffer pH 7.8, and squeezing through muslin. The alcohols were obtained from the Eastman Kodak Co. They included ethanolamine, diethanolamine, β-β'-dihydroxyethyl ether, β-β'-dihydroxyethylsulfide, ethylene bromohydrin, tribromoethanol (avertin), glycerol, ethylene chlorohydrin, ethylene glycol, and β-hydroxypropionitrile. The last 3 were the only ones oxidized by the liver preparation. Figure 1 shows the oxidation of various concentrations of these 3 compounds compared with the oxididation of ethyl alcohol. The Schiff test

[4] H. L. Gillum, unpublished data.

* One of us (P. H.) is indebted to the John and Mary R. Markle Foundation for its support during this investigation.

[1] Lutwak-Mann, C., *Biochem. J.*, 1938, **32**, 1364.

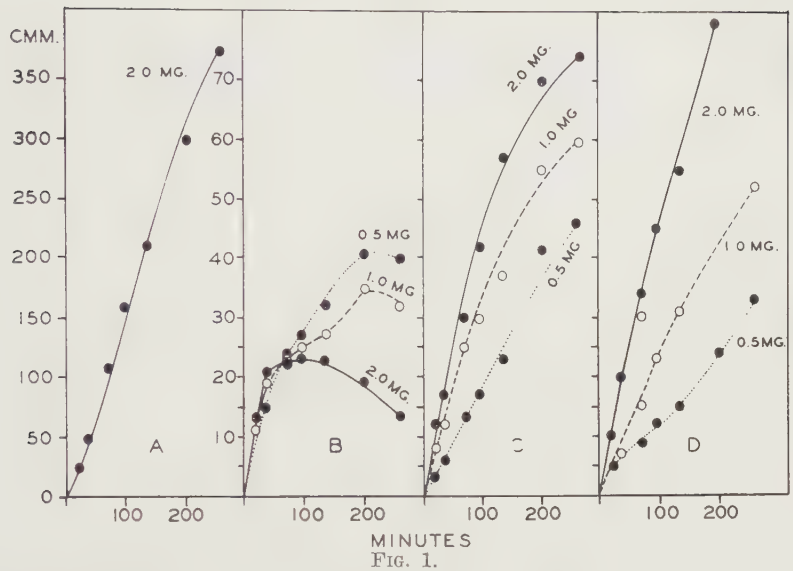

FIG. 1.

The oxidation of various alcohols by rat liver suspension, pH 7.8, 37°. The oxygen uptake of liver alone has been subtracted from the uptake of liver and alcohol. A--ethyl alcohol; B—ethylene chlorohydrin; C-β—hydroxypropionitrile; D—ethylene glycol.

showed that aldehydes were present in concentrations greater than in the control when these compounds were oxidized. The aldehyde formed from ethylene chlorohydrin is evidently toxic because it inhibits the control oxygen uptake of the liver. Tissues lacking the alcohol oxidase, *i. e.*, brain and kidney, fail to oxidize the substituted compounds. None of the compounds inhibit the oxidation of ethyl alcohol. 0.005 M KCN inhibits the oxidations. The pH optimum for the oxidation of the substituted compounds is the same as that for ethyl alcohol.

Summary. Of a series of substituted ethanols and ethanol derivatives, only ethylene chlorohydrin, ethylene glycol, and β-hydroxypropionitrile are oxidized by liver preparations containing an active alcohol oxidase.

12032

Variations in Arginase Concentration in Livers of White Rats Caused by Thyroxine Administration.

HOWARD D. LIGHTBODY, EWALD WITT AND ABRAM KLEINMAN.
(Introduced by D. M. Greenberg.)

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The literature concerning stored and ingested materials as energy sources in the increased rate of metabolism caused by thyroxine administration has been reviewed by Kendall[1] and Harington.[2] Nitrogen excretion after administration of thyroxine, or desiccated thyroid, has been found not to be in direct relation to changes in the basal metabolic rate. Protein breakdown is said to be initially accelerated by the hormone. Continued administration, however, appears to be accompanied by decreases in nitrogen output, to, or even below, the initial level. Nitrogen equilibrium can be maintained at times of high basal metabolic rate by medication on very low daily protein intakes, and by providing sufficient carbohydrate and fats to meet caloric requirements. The quantities of nitrogen excreted and protein catabolized are dependent upon the carbohydrates and fats available. Changes in nitrogen metabolism caused by thyroxine, therefore, depend upon caloric intake and type of food in relation to requirements.

Bodansky and Duff[3] have found pregnant rats have a marked tolerance to thyroxine. Daily administration of one milligram of the hormone during the last 10 to 12 days of gestation failed to interfere with gains in weight as compared with pregnant controls. Danforth and Loumos[4] have found that in the case of female rats at constant food intakes after feeding desiccated thyroid the increase in rate of oxygen consumption by pregnant animals is less than that by nonpregnant controls. Pregnant rats were able to maintain the rate of increase in weight on doses that caused marked decreases in nonpregnant rats.

Analysis of livers of white rats[5] after feeding at various levels

[1] Kendall, E. C., *Thyroxine*, The Chem. Catalog Co., New York, 1929.

[2] Harington, C. R., *The Thyroid Gland, Its Chem. and Physiol.*, Oxford University Press, London, 1933.

[3] Bodansky, M., and Duff, V. B., *Endocrinology*, 1936, **20**, 537.

[4] Danforth, D. N., and Loumos, S., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 870.

[5] Lightbody, H. D., and Kleinman, A., *J. Biol. Chem.*, 1939, **129**, 71.

of protein intake have shown that the concentration of arginase in the livers, and the total arginase per unit of body weight, varies directly with the quantity of protein ingested. Similar analysis[6] of livers from fasted rats indicated a correlation between the quantities of arginase in the livers and the type of body materials, carbohydrates and fats, or protein that serve as primary energy sources at the time of sacrifice. Sex differences[7] in concentration of the enzyme characteristic of the reproductive period therefore seem likely to be related to sex differences in protein metabolism.

The object of the experiments reported here was to study the action of thyroxine upon the concentration of liver arginase when administered to male and to pregnant and nonpregnant female rats.

Procedure. One milligram of crystalline thyroxine was administered by subcutaneous injection daily, for 9 days, to rats of 3 groups. These groups consisted of from 8 to 10 animals each and were made up of males (I), nonpregnant females given thyroxine (II), and pregnant females given thyroxine (IV). A similar group (III) of pregnant animals was used as controls. The periods of injection were so selected that sacrifice was made during the 17th or 18th day of pregnancy and when the ages were 96 to 106 days. These are the approximate ages of greatest sex differences in concentration of arginase in the liver.[7] Daily records of food consumption of animals in Groups I and II were kept for a preperiod of 5 days and during the 9 days of thyroxine injection. Similar records were kept for pregnant animals of Groups III and IV only during thyroxine administration. The housing and feeding of the animals, and the methods of collection of tissue, and determination of arginase have been described elsewhere.[7] A summary of the results is given in Table I.

Discussion. The data show that the responses of the sexes to thyroxine injection were quite different. Of the 10 males injected (Group I) all but 1 lost weight. The average loss amounted to 8.8% of the average initial body weight. Three nonpregnant females in the group of 10 (Group II) given thyroxine lost some weight but the average change amounted to a gain of 2%. Food intake by the males was decreased during the treatment. The food intake of the nonpregnant females was not changed by thyroxine injection. The thyroxine-treated pregnant rats of Group IV did not show a marked change in food intake when compared with controls (Group

[6] Lightbody, H. D., and Kleinman, A., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 25.

[7] Lightbody, H. D., *J. Biol. Chem.*, 1938, **124**, 169.

TABLE I.
Summary of Analytical Data.
Except in first 3 columns values given are group means.

Group No.	No. in Group	Sex	Body wt, gain or loss, %	Uterus and fetuses, total wt, g	Liver			
					Solids, %	Dry, per 100 g rat, g	Arginase units*	
							Per mg dry liver	Per 100 g rat $\times 10^{-3}$
I	10	M	— 8.8	—	30.8±0.2	1.06±0.02	243.9±8.5	259.9±10.1
II	10	F	+ 2.0	—	30.4±0.3	1.23±0.02	223.9±6.6	273.9± 6.8
IV	10	F(P)	+22.3	22.0	29.7±0.2	1.29±0.02	232.3±5.2	303.5± 8.6
			Controls (uninjected).					
V†	18	M	—	—	33.7±0.2	1.15±0.02	219.3±7.2	252.9± 9.5
VI†	12	F	—	—	33.8±0.4	1.18±0.03	176.9±4.2	207.8± 4.7
III	8	F(P)	+29.9	22.9	32.8±0.3	1.36±0.05	174.9±6.4	237.9±12.0

*The arginase unit is that quantity of the enzyme which will liberate urea equivalent to one micromole (M $\times$ 10^{-6}) of carbon dioxide under the experimental condition used.

†The values for these controls are those obtained after feeding a diet containing 25% of milk proteins. These results have been previously reported[5] and are repeated here for convenience.

The average daily food consumptions in grams per 100 g body weight per day were as follows:

Group I, preperiod 6.5, thyroxine injection period 4.9.
Group II, preperiod 6.0, thyroxine injection period 6.0.
Group III, preperiod —, control period 7.3.
Group IV, preperiod —, thyroxine injection period 6.9.

III) during a similar period of gestation. The livers from all groups of rats (I, II, IV) given thyroxine were found to have lower percentage of solids than did those from non-treated groups (III, V, VI). The quantity of dry liver tissue per 100 g of body weights was found to be greater in pregnant rats than in others of the same sex. The administration of thyroxine was found to have not significantly changed this value. Neither did the hormone cause loss, or changes in weight of the fetuses and associated tissues.

The quantity of total arginase in livers of male rats given the hormone (Group I) was not different from that in livers of similar animals fed a diet near the optimum in protein content (Group V). Unlike male rats fasted for 2 days,[6] which had lost a comparable percent in body weight, the thyroxine-treated males maintained the quantity of total liver arginase at levels characteristic of well fed animals. Thyroxine administration to male rats did not cause marked increases in liver arginase as did the feeding of diets high in protein.

Livers of pregnant rats (Group III) were found to contain more arginase than those from the nonpregnant controls (Group VI). The increase was due to the greater size of the organ rather than to a difference in concentration. The arginase concentration and total arginase in the liver of the nonpregnant female rats given

thyroxine (Group II) were found to be considerably greater than in the livers of the Group VI controls and to have become comparable to similar values found in livers of male rats (Group V) after feeding a diet of near optimum protein content. Similar changes in arginase values in the livers of female rats have been found after feeding high protein diets[5] and after fasting.[6] High protein feeding, as previously reported,[5] caused increases both in concentration of the enzyme and in size of the organ. Fasting caused increases in concentration but liver sizes were decreased. Pregnant rats given thyroxine (Group IV) did not increase in body weight as much as did the pregnant controls (Group III). They did, however, increase both the quantity of the liver tissue and the concentration of the enzyme. The values found for liver size, concentration, and total quantity of enzyme, upon analysis of the livers of these animals are closely comparable to those previously found for livers of female rats fed diets containing 75% protein for 21-25 days.

If the quantity of liver arginase per unit of body weight is used as an index of the demands placed upon the enzyme system concerned with protein catabolism, the metabolic responses of male and female rats to thyroxine administration are indicated by these data to be different. Livers from treated males contain quantities of arginase quite comparable to those found in untreated males. It seems likely, therefore, that any readjustment in enzyme systems caused by the hormone must have been made primarily in systems other than those concerned with protein catabolism. The quantities of the enzyme found in livers of female rats, both pregnant and nonpregnant, are similar to those found in livers of female rats fed diets high in proteins, or after fasting for long periods. It seems likely, therefore, that a readjustment in the enzyme system concerned with protein catabolism is caused by thyroxine when injected into female rats.

Summary. Analysis of the livers of white rats for arginase activity after the administration of thyroxine have been made. The data show that, although the hormone in the dosage used caused male rats to lose body weight, the arginase content of the livers remained unchanged. Livers from pregnant and nonpregnant female rats given thyroxine contained quantities of the enzyme comparable to those found after feeding diets high in protein or after long fasting. Possible relations of these changes in enzyme concentration to protein catabolism are discussed.

12033 P

Treatment of Lead Poisoning with Sodium Citrate.

Seymour S. Kety and T. V. Letonoff. (Introduced by J. Harold Austin.)

From the Biochemical Laboratory, Philadelphia General Hospital.

In investigations undertaken by one of us (S.S.K.) it was found that sodium citrate in dilute solution exerts a powerful solvent effect on tertiary lead phosphate,[1] that citrate removes lead ion from solution by the formation of a soluble complex of extremely low dissociation,[2] and that the administration of sodium citrate to lead poisoned rats produced a significant increase in the excretion of lead.[1] The junior author (T.V.L.) independently observed that in a case of plumbism receiving potassium citrate incidental to a chloride excretion test there was a sharp drop in the blood lead level and a rise in the urinary output of lead. (Chart I, No. 7.) These observations led to the present study of the effects of the administration of sodium citrate in plumbism.

Six cases of lead poisoning seen at the Philadelphia General Hospital since August, 1940, provided the basis of this study.* All presented clinical evidence of mild to severe lead intoxication and all had abnormally high lead concentrations in whole blood on admission. Each adult received from 2 to 4 g of sodium citrate and the children from 1 to 2 g by mouth 3 times daily. Blood lead concentrations and, in 3 cases, urinary lead excretions were determined before and at intervals during the course of treatment by the method of Letonoff and Reinhold[3] slightly modified.[4]

The results are presented in Chart I. There was a rapid disappearance of toxic symptoms without untoward reactions in every instance and the blood lead concentration fell to normal or nearly normal levels, (normal range as determined by this method is from 0 to 0.05 mg lead per 100 g of whole blood). Urinary excretion studies gave variable results with a trend toward increased urinary excretion of lead during treatment. In Chart II are pre-

1 Kety, S. S., read before the Undergraduate Medical Association, Medical School, University of Pennsylvania, April 4, 1940.

2 Kety, S. S., to be published.

* The authors are indebted to Dr. W. Brody, Dr. E. L. Noone, the late Dr. R. H. Thompson, the late Dr. R. G. Torrey, and Dr. L. A. Wikler on whose services these patients were treated.

3 Letonoff, T. V., and Reinhold, J. G., *Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 280.

4 Letonoff, T. V., to be published in *Ind. Eng. Chem., Anal. Ed.*

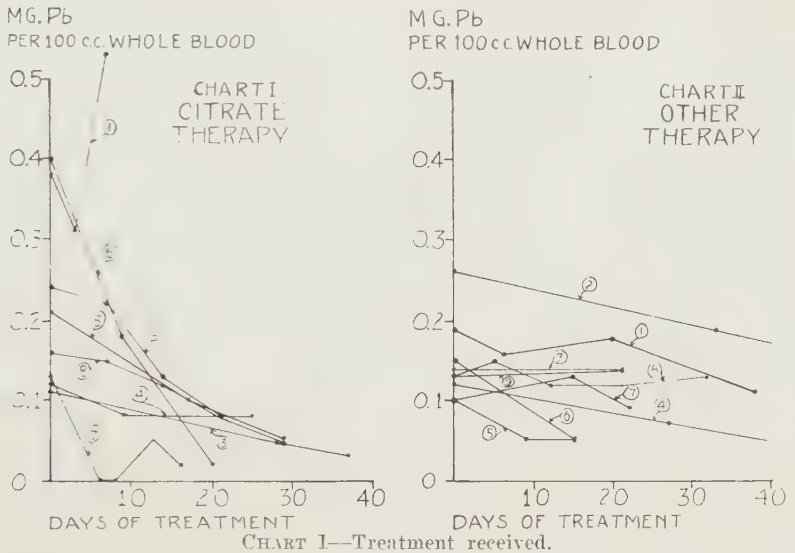

CHART I—Treatment received.

1. Sodium citrate throughout.
2. (Readmission of No. 1 after 47 days without treatment) Sodium citrate throughout, calcium carbonate 14th to 21st day.
3. Sodium citrate throughout.
4. Sodium citrate, iron and copper and thiamin chloride throughout.
5. Sodium citrate throughout, calcium gluconate 17th to 29th day.
6. Sodium citrate throughout.
7. Potassium citrate 2d and 3d days.
8. Sodium citrate throughout.

CHART II—Treatment received.

1. Disodium phosphate, cevitamic acid and oleum percomorphum throughout.
2. Disodium phosphate, diet high in phosphorus and calcium, viosterol, cevitamic acid and thiamin chloride throughout.
3. Oleum percomorphum throughout.
4. Calcium lactate and calcium phosphate throughout, cevitamic acid 9th to 14th day, cod liver oil 14th to 57th day.
5. No specific treatment.
6. Calcium phosphate 5th to 15th day.
7. Calcium chloride throughout.
8. Dihydrotachysterol throughout.
9. (Readmission of No. 8 after 10 months without treatment) Brewer's yeast, nicotinic acid and cevitamic acid throughout, monosodium phosphate and calcium carbonate 6th to 21st day.

sented the changes in blood lead (determined by the same method) of patients with plumbism receiving other therapy.

A controlled study is in progress with other citrate compounds to determine the means whereby lead is removed from the blood. Whatever the mechanism of action of sodium citrate, the rapid alleviation of symptoms and the drop in blood lead levels observed should make it a useful therapeutic agent and a possible prophylactic measure in lead poisoning.

The authors wish to express their gratitude for the guidance of Dr. John G. Reinhold.

12034

Experimentally Produced Wounds as a Route of Fatal Infection Caused by *Bacillus violaceous.**

Herbert J. Schattenberg and David Van Brown. (Introduced by C. W. Duval.)

From the Department of Pathology, Tulane University, New Orleans, Louisiana.

Although *B. violaceous* is generally considered as of saprophytic and non-pathogenic nature, it is evident that at least certain strains of this microörganism demonstrate virulent and fatal infection for man and lower animals. Woolley[1] demonstrated such infection with rapid production of death in carabao or water-oxen. Black and Shahan[2] and Soule[3] reported protracted infections in the human subject which eventually proved fatal. In the present human case to be reported[4] infection, septicemia, and death within a week were noted in a robust adult who previously had enjoyed excellent health.

In a review of the aggregate of the instances wherein fatal *B. violaceous* infection had occurred there is reason to believe that the microörganism had gained entrance through injury or wounds of the extremities. In the case herein concerned, there was a definite history of infection through a splinter wound of the foot with pain of this extremity and accompanying adenitis. While consistent pathogenicity for lower animals has been reported for the culture isolated,[5] the microörganism was injected by use of a syringe through the subcutaneous, intraäbdominal or intravenous route.

The present experiments were undertaken to ascertain if infection with *B. violaceous* could occur through surface injury. Varied types of wounds were produced in the feet of white mice and rabbits with subsequent exposure to infection by this microörganism, thereby mimicing to a reasonable degree the condition considered as the natural atrium of bodily invasion.

White Mice. The soles of the hind feet of 10 such animals were scarified to a depth well beneath the skin by use of a scalpel. Subsequently, the wounded parts were either dipped into or swabbed with

* Aided by a grant from the David Trautman Schwartz Research Fund.

1 Woolley, P. G., *Johns Hopkins Bull.*, 1905, **16**, 89.

2 Black, M. E., and Shahan, J., *J. A. M. A.*, 1938, **110**, 1270.

3 Soule, M. H., Abs. Scientific Proc. Am. Assn. Pathologists and Bacteriologists, *Am. J. Path.*, 1939, **15**, 592.

4 In press.

5 Schattenberg, H. J., Proc. Soc. Exp. Biol. and Med., 1940, **45**, 829.

a dilution of broth culture of *B. violaceous* contained in Petri dishes. This exposure to infection lasted for approximately a minute and was repeated 24 hours later, although the wounds at that time presented "scabbing" or crusted coagulum. All of these animals showed tumefaction and impaired function within 24 hours in the leg wherein the sole was traumatized. Within 4 to 6 days, 8 of the infected animals were very perceptibly sick. As death was impending, blood cultures were procured from the heart in 4 of the animals and all yielded heavy growths of *B. violaceous*. These animals died from the heart puncture while the 4 remaining mice survived for an additional 24 to 48 hours. Necropsies of these animals showed inguinal adenitis of varying degrees apparently contingent upon host resistance or the number of invading microörganisms. In other words, those animals surviving for the longer periods revealed a more marked adenitis. The glands were congested and swollen and in 2 instances, small areas of necrosis were noted. In the abdominal cavity, the retroperitoneal nodes presented similarly varying degrees of involvement. Multiple very minute light yellow lesions were found especially in the liver and at times in the spleen and lungs. The microscopic study of the involved structures presented necrosis of the parenchymatous cells with, at times, peripheral cellular response of the lymphoid, plasma and neutrophilic varieties. Cultures of the gross lesions and heart's blood yielded heavy growths of *B. violaceous*.

Controls. Ten white mice having normal or unabraided feet were submitted to similar contact with the *B. violaceous* suspension. These animals remained normal and unaffected.

Rabbits. Twelve such animals were employed. In 5 of these, the fur was clipped on one hind foot and a small incision was made through the skin and into the subcutaneous structure. To simulate an infected splinter, a portion of a wooden tooth-pick 6 mm in length, which had been dipped in a broth culture of *B. violaceous,* was introduced into the tissue at the upper end of the wound, parallel to the line of incision. For 3 other rabbits, after clipping the fur on the sole of one rear paw, one incision approximately 1 cm in length with 2 to 3 smaller cross incisions were made, all of which extended into the subcutaneous tissue. These incised areas together with the paws of 4 others wherein no incision was made were swabbed with a broth culture of *B. violaceous* for approximately a minute on 2 successive days.

The 5 rabbits having infected "splinters" in the feet showed within 24 hours swelling and dysfunction of the corresponding leg.

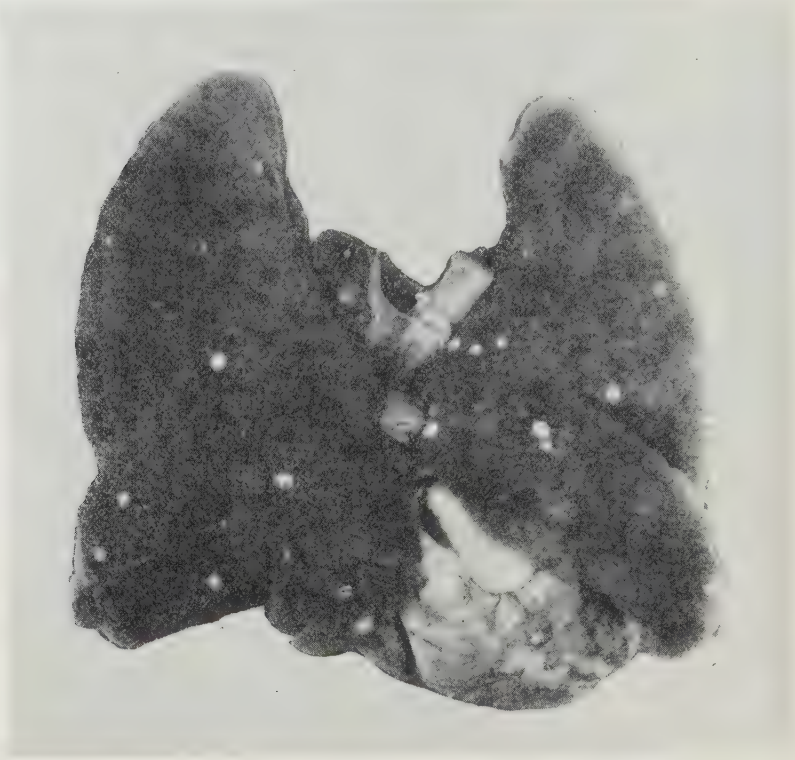

Fig. 1.

The lungs show numerous necrotic nodules of varying size and shape scattered beneath the pleural surfaces. A similar lesion is noted on the anterior surface of the heart, shown below.

They became perceptibly and progressively ill. One of these died within 4 days. At necropsy the leg was swollen but the inguinal nodes showed nothing noteworthy. The lungs contained scattered small yellow lesions measuring approximately 1 mm in diameter (Fig. 1.) A few such lesions were noted in the liver and heart wall. Microscopic study revealed similar findings as those described for the mice but to a more exaggerated extent especially in the liver. (Fig. 2.) In the lungs, marked congestion, oedema and mild pneumonitis occasionally surrounded the necrotic foci. Cultures made from the heart's blood and the lesions of the lungs, liver and feet yielded growths of *B. violaceous*. Of the 4 remaining rabbits with "splinters" one died in 8 and one in 9 days, the 2 others have survived. The necropsies of these animals showed in general the same aspects as that described for the above rabbit. There was, however, marked inguinal adenitis with some necrosis of structure.

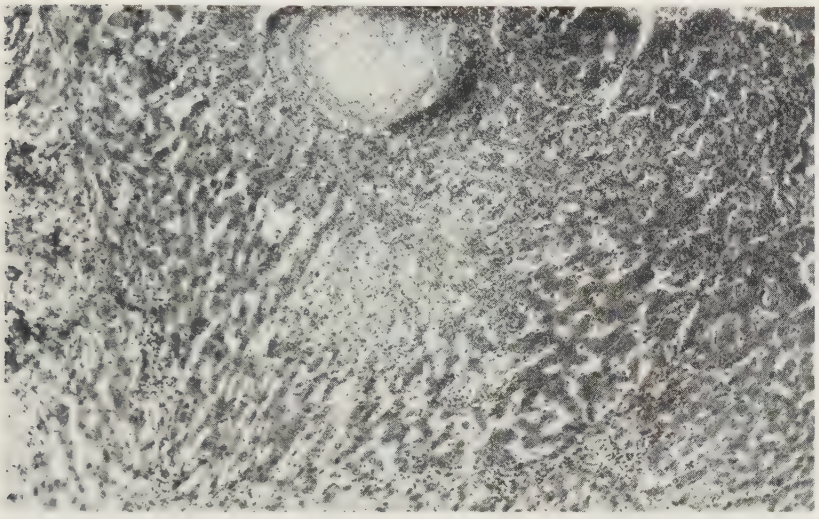

Fig. 2.

In this section of the liver there is seen a necrotized oval nodule. Below this is shown an earlier lesion of focal necrosis in which pyknosis and karyorrhexis of the nuclei has occurred.

Of the 3 rabbits wherein the crucial incisions were submitted to *B. violaceous* infection, one died in 11 days and one in 2 weeks. The remaining animal has survived. Necropsy of the 2 animals showed scattered small gross lesions in the lungs and liver. In one animal a marked inguinal adenitis was present with congestion and areas of necrosis. One of the glands was enlarged to approximately 1 cm in the longest diameter. The microscopic study was similar to that already described. Cultures of the lesions and heart's blood yielded growths of *B. violaceous.*

The 4 rabbits wherein no injury was present in the feet, although submitted to similar bacterial exposure as those with incisions, remained unaffected and normal.

Route of Infection. In these experimental injuries or wounds the invasion of *B. violaceous* must occur, through the lymphogenous or hematogenous routes or both. The marked adenitis on the one hand indicates a lymph stream invasion finally traveling to the blood circulation as shown by positive blood cultures. On the other hand the lack of notable lymph gland involvement and the accentuated extent of lesions in the lung in certain of the animals, suggest direct primary blood circulatory ingress as the principal route of infection. In either event, the likelihood and rapidity of invasion through such routes by this microörganism is clearly demonstrated.

Summary. It is shown herein that infection with *B. violaceous* may readily occur through various types of surface injury of the feet of animals. It is suggested, therefore, that ingress through external injury forms the usual portal of entry of *B. violaceous* in the natural infection. These infections are fulminating and usually fatal in a short period of time. In this connection, it appears probable that both the lymph and blood streams may form the routes of host invasion although the lymphatic pathway is emphasized. The lesions produced are similar to those found in the natural infection of carabao as well as those observed in the human infection. Although *B. violaceous* has in general been regarded as innocuous, it is further stressed herein that certain strains are definitely pathogenic and present evidence of distinct virulence. Furthermore, a likely portal of entry of the natural infection is indicated.

12035 P

Nutrition of *Tetrahymena geleii* (Protozoa, Ciliata).

VIRGINIA C. DEWEY.* (Introduced by C. A. Stuart.)

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All attempts to grow *Tetrahymena geleii* in a medium of known composition have, so far, failed. Failure to obtain growth in anything but peptones led Lwoff[1] to formulate the hypothesis that the ciliate requires polypeptides and cannot utilize free amino acids. Earlier[2] he had hinted that the failure of growth in a mixture of silk peptone, fibrin peptone and gelatin, which contains all the known amino acids, is the result of the lack of some supplementary substance. Gelatin[3] will support growth if supplemented with a small amount of yeast extract; indeed, it is stated[4] that transplantable growth is obtained in gelatin plus thiamin and riboflavin. Silk peptone gives slight, but transplantable, growth if thiamin is added.[5]

* Aided by a grant from the Manufacturers' Research Fund for Bacteriology and Protozoology at Brown University.

1 Lwoff, A., *Monogr. de l'Inst. Pasteur*, 1932.

2 Lwoff, A., *C. R. Soc. Biol.*, 1924, **91**, 344.

3 Hall, R. P., and Elliott, A. M., *Arch. f. Protistenk.*, 1935, **85**, 443.

4 Hall, R. P., *Anat. Rec.*, 1940, **78** (suppl.), 164.

5 Lwoff, A., and Lwoff, M., *C. R. Soc. Biol.*, 1937, **126**, 644; D. M. Lilly, personal communication.

It is clear, then, that the question of the amino acid requirement of *T. geleii* cannot be answered until more is known of the supplementary needs.

In order to discover the vitamin (or supplement) requirements, a basal medium is necessary. Since it had been found that the ciliate will grow well in a 1% solution of crude casein (Eimer and Amend), a similar solution of Casein-Harris (free from fat and water soluble vitamins) was tested. It gave no growth beyond the first or second transplant.

Slight improvement in growth was noted upon the addition of thiamin to this medium. All possible combinations of thiamin, riboflavin, nicotinic acid, pyridoxin, pantothenic acid, biotin (as a concentrate) and inositol† were added. Growth was better in the media containing riboflavin and pyridoxin in addition to thiamin, but it was slow and never reached the concentration obtained in crude casein. It was evident that some other factor is required for optimum growth. The possibility of toxicity of the medium was eliminated, since good growth occurred upon the addition of 0.1% yeast concentrate (Yeast Vitamin Harris) to the vitamin-free casein, whether or not other supplements were present.

The lacking growth factor was found to be present in various yeast preparations (whole, killed cells; extract; autolysate and concentrate), egg yolk and water extract of timothy hay. After a number of unsuccessful attempts to free the yeast and egg preparations from protein and protein degradation products in order that the nitrogen requirements of the organism could be studied, attention was turned to the hay extract.

The crude hay extract was prepared by boiling timothy hay in distilled water and filtering to remove particles. The filtrate is very low in protein content. Upon addition of the extract to vitamin-free casein plus thiamin, excellent growth results. Addition of normal lead acetate to the extract gives a precipitate. After filtration lead is removed from both fractions with phosphoric acid. All the protein remains in the filtrate. When added to vitamin-free casein neither fraction alone gives optimal growth, but the 2 added together give growth equal to that of the controls. The filtrate fraction may be treated with alcohol to remove the protein. A precipitate is obtained which is devoid of activity. After removal of the alcohol, the filtrate was found to be active when added to the lead precipitate fraction.

† Thiamin and riboflavin: Hoffmann-La Roche; nicotinic acid and inositol: Eastman Kodak; pyridoxin and pantothenic acid: Merck; biotin: S. M. A. Corp.

There are, then, at least 2 factors required for the growth of *T. geleii* in addition to thiamin and possibly riboflavin or other known vitamins. Further attempts at purification of these factors are in progress. Meanwhile, the protein-free hay fractions are being used to determine the amino-acid requirements of the organism.

Gelatin (vitamin-free Harris) was made up in 1% solution containing the 2 fractions from hay plus thiamin and pyridoxin. While growth was obtained in the sixth transplant in this medium, it is far from equal to that in crude casein. No growth at all occurred in second transplant in a solution containing the ten essential amino acids (for the rat) in the proportions found in 1% casein. Both of the hay fractions, thiamin and pyridoxin were also present. There are 5 possible reasons for the failure of the organism to grow in the amino acid solution: 1. Polypeptides are required; 2. some supplement is lacking; 3. an essential amino acid is lacking; 4. the medium contains a toxic substance; 5. the osmotic pressure of the medium is too high. This last possibility seems the most likely, since toxicity is eliminated by the fact that the addition of 1 ml of the amino acid solution to 5 ml of 1% gelatin plus supplements makes possible excellent growth.

12036

Concentration of Free Sulfanilamide, Sulfapyridine and Sulfathiazol in Material Drained from Human Biliary Tract.

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Two grams of sulfanilamide, sulfapyridine and sulfathiazol were ingested in successive experiments by 4 patients from whom bile was draining through tubes inserted in the bile duct after operations upon the biliary tract. Intervals of 3 or 4 days separated the experiments upon each subject. A satisfactory test with sulfapyridine was not obtained in one instance. Specimens of bile were collected for 4 successive 4-hour periods in each experiment. The first period preceded, and the others followed the ingestion of the drug. Samples of urine were collected simultaneously with the bile specimens after the drug had been given, and a sample of blood was drawn at the midpoint of each of these 3 4-hour periods. The free compounds were determined in the specimens of bile by the diazo technic of Brat-

ton and Marshall[1] in the presence of acetone. Since the usual methods for clarifying solutions, including the one used upon bile by Hubbard and Anderson[2] precipitated a rather high proportion of added sulfathiazol, the bile was clarified by the use of 2 successive precipitations with barium—first in alkaline solution as the phosphotungstate and then in acid solution as the sulfate. The sulfonamide drugs could be determined with an accuracy of about 5%, or, if present in very low concentrations, to the nearest 0.1 mg per 100 cc by this procedure. Control specimens, analyzed as a part of each experiment, gave no red color by the technic. The concentrations of the drugs in the urine were determined after dilution, one part to 100, by the same diazo technic. Blood analyses were carried out as described by Bratton and Marshall.[1]

The results upon the blood and bile of different subjects were qualitatively similar, except for such variations in actual blood concentrations as would be expected from the varying ease of absorption of the different compounds studied.[3] The actual relationships between the concentrations in blood and bile were sufficiently alike in the studies upon the different patients to justify presenting an average of the figures. Although the excretion in the different experiments varied rather markedly, corresponding, at least in some instances, with the excretion of total fluid,[4] the results have also been com-

TABLE I.
Excretion of Free Sulfonamide Compounds.
Average for 3 patients.

Compound	Period, 4 hr	Blood, mg/100 cc	Bile, mg/100 cc	Urine	
				Vol., cc	Drug, mg
Sulfanilamide	1st	5.5	3.6	359	102
	2d	3.9	2.6	181	100
	3d	2.8	2.3	250	111
Sulfathiazol	1st	2.9	0.1	115	102
	2d	3.1	0.3	197	131
	3d	2.1	0.4	417	217
Sulfapyridine	1st	1.4	0.7	387	26
	2d	1.4	1.3	212	55
	3d	1.2	1.1	230	44

[1] Bratton, A. C., and Marshall, E. K., Jr., *J. Biol. Chem.*, 1939, **128**, 537.

[2] Hubbard, R. S., and Anderson, R. K., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 487.

[3] Goodman, L., and Gilman, A., *The Pharmacological Basis of Therapeutics*, New York, 1941, pp. 1014, 1068, 1087.

[4] Marshall, E. K., Jr., Emerson, K., Jr., and Cutting, W. C., *J. Pharm. Exp. Therap.*, 1937, **61**, 191.

bined. In Table I are given the average values for the 3 patients who received all 3 of the compounds studied.

The table shows the lack of a quantitative relationship between the amounts of the drugs present in the blood and urine which has repeatedly been demonstrated, and suggests, particularly in the experiments upon sulfanilamide, that the rate of excretion of these substances is relatively constant when the urine volume is constant.[4] The excretion of the drugs into the bile shows marked qualitative differences, which, as already stated, was present in each of the individual experiments. Considerable amounts of sulfanilamide were present in the bile. These paralleled approximately the concentrations in the blood, but the values of the ratio $\frac{\text{Free sulfanilamide in bile}}{\text{Free sulfanilamide in blood}}$ increased during the later parts of the experiments as previously reported.[2] The concentration of sulfathiazol, in the bile, on the other hand, was very low throughout the periods of study, and increased rather than diminished in spite of the drop in the concentration in the blood. This increase in concentration, although slight, was much beyond the limit of accuracy of the methods, and was shown in all the experiments carried out. The results with sulfapyridine were more irregular than were those obtained with the other two drugs, and perhaps are associated with the effect of the known irregularities in the absorption of this compound[5, 6] upon the blood concentrations. The figures obtained in these studies resemble, the authors believe, those obtained with sulfanilamide rather than the results with sulfathiazol.

The results with sulfathiazol are difficult to interpret. It has been reported that this drug enters the spinal fluid in relatively low concentration but diffuses fairly readily into other body fluids.[7] It is possible that this relative failure of passage into the liver is an expression of a general relatively low diffusibility of this compound. Against such an assumption are the figures upon the urinary excretion of this substance shown in the table and included in the literature.[8] It is possible that there is a precipitation or alteration of the compound involved in the loss, but if this is so the loss must be during the formation of the bile, for no significant change in the

[5] Long, P. H., and Feinstone, W. J., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 486.

[6] Brown, W. H., Thornton, W. B., and Wilson, J. S., *J. Clin. Invest.*, 1939, **18**, 803.

[7] Sadusk, J. F., Jr., and Hershfeld, J. W., quoted by Goodman, L., and Gilman, A., *The Pharmacological Basis of Therapeutics*, New York, 1941, p. 1088.

[8] Sadusk, J. F., Jr., Blake, F. G., and Seymour, A., *Yale J. Biol. Med.*, 1940, **12**, 681.

sulfathiazol content occurred when bile was stored for several days. It was not possible to test the material for the presence of acetylated sulfathiazol by the methods available. It seems improbable although not absolutely impossible, that acetylsulfathiazol might have been present in bile in spite of the practically complete absence of acetylsulfanilamide in such material.[2] The authors are not able to give a satisfactory interpretation of this finding, but they do wish to point out one result of the difference in concentration of sulfanilamide and sulfathiazol in the bile. When relatively large amounts of a readily absorbed substance enter the bile and are returned to the intestine the time during which the compound remains in the body is prolonged. Excretion by the liver, therefore, will tend, at least slightly, to prolong the period during which sulfanilamide remains in the body as compared with the time sulfathiazol remains in the body.

12037

Ineffectiveness of Adrenal Cortical Hormone on Serum Complement of the Guinea Pig.*

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Partial adrenalectomy increases the susceptibility of guinea pigs and rats to anaphylactic shock.[1, 2, 3] The lethal dose of antigen has been found to be inversely proportional to the degree of adrenal insufficiency. Wolfram and Zwemer[4] report that "cortin" protects normal sensitized guinea pigs against fatal anaphylaxis. In the dog the severity of anaphylactic shock is said to be lessened by the previous injection of cortical hormone.[5] Perla and his coworkers[6]

* The expenses of this investigation were provided by the Proctor Fund, Harvard University.

1 Kepinow, L., *Compt. rend. Soc. Biol.*, 1922, **87**, 327.

2 Flashman, D. H., *J. Inf. Dis.*, 1926, **38**, 461.

3 Wyman, L. C., *Am. J. Phys.*, 1929, **89**, 356.

4 Wolfram, J., and Zwemer, R. L., *J. Exp. Med.*, 1935, **61**, 9.

5 Dragstedt, C. A., Mills, M. A., and Mead, F. B., *J. Pharm. and Exp. Ther.*, 1937, **59**, 359.

6 Perla, D., Freiman, D. G., Sandberg, M., and Greenberg, S. S., Proc. Soc. Exp. Biol. and Med., 1940, **43**, 397.

report an increased resistance in rats to histamine shock by the use of "cortin". These observations parallel others in which cortical extract is said to improve the lowered resistance of adrenalectomized animals to bacterial infection.[7, 8] This has been explained as due to increased antibody production, especially in the earlier stages of the immunizing process.[9]

Since anaphylactic shock, whether actively or passively transmitted, is accompanied by a fall in serum complement,[10] it is of interest to see whether the reported protective effect of cortical hormone can be attributed to an alteration in the titer of the serum complement.

Method. Healthy adult guinea pigs were kept on a high vitamin C diet for several weeks prior to use, in order to exclude any effect of vitamin C deficiency on the level of serum complement.[11] Blood for titration of complement was obtained by cardiac puncture, with or without ether anesthesia. (In a series of preliminary experiments the use of ether anesthesia did not change the complementing activities of the sera. This confirms the observations of Ecker.[12]) The method of titration of complement was exactly as previously reported.[13] Sera were diluted 1:30 with Brooks' solution and titrated immediately after dilution for the point of initial hemolysis.

Two different preparations of cortical hormone were used: (1) adrenal cortical extract in 10% alcohol (50 dog units per cc) "Cortin"† and (2) desoxycorticosterone acetate in sesame oil (5 mg per cc) "Percorten."‡ Control animals were used for complement titrations in all experiments.

Twenty animals were injected intraperitoneally with varying amounts of Cortin (5 to 150 dog units) or Percorten (0.5 to 8.0 mg) and bled from 2 to 6 hours later. Control animals were injected with the same amount of sterile saline solution. The complement titer of the blood at the point of initial hemolysis was not significantly different in the treated group (0.045 cc of serum) from

[7] Perla, D., and Mormorston, J., *Arch. Path.*, 1933, **16**, 379; *Endocrinology*, 1940, **27**, 367.

[8] Zinsser, H., Enders, J. F., and Fathergill, L. G., *Infection, Immunity and Resistance*, Macmillan, 1939.

[9] Fox, C. A., and Whitehead, R. W., *J. Immunology*, 1936, **30**, 51.

[10] Zinsser, H., and Jones, S. Bayne, *Textbook of Bacteriology*, 7th ed., Appleton-Century Co., 1934.

[11] Ecker, E. E., Pillemer, L., Wertheimer, D., and Gradis, H., *J. Immunology*, 1938, **34**, 19.

[12] Ecker, E. E., Pillemer, L., and Wertheimer, D., *J. Immunology*, 1938, **34**, 39.

[13] Cope, O., and Kapnick, I., *Endocrinology*, 1940, **27**, 533.

† Kindly furnished by Upjohn & Co.

‡ Kindly furnished by Ciba & Co.

that of the control group (0.041 cc of serum). This was true even with massive doses (up to 500 dog units of Cortin and 30 mg of Percorten). No immediate or late ill effects from these substances were noted.

A group of 5 animals were injected with 0.5 mg of desoxycorticosterone acetate 3 times daily for 10 days, and complement titrations were carried out before starting the experiment, after the fifth day, and also after the tenth day in the 4 surviving animals. One animal died as a result of cardiac puncture. No change in the serum complement titer was observed in any of these animals.

In further experiments, in which the cortical extract was added to guinea pig sera *in vitro*, no change in complement was demonstrated.

Five animals were sensitized to horse serum by the intramuscular injection of 0.1 cc of horse serum. Complement titrations carried out 14 days later showed no significant change after a period of 2-6 hours following the intraperitoneal injection of cortical extract in doses similar to those listed above.

Comment. Cope and Kapnick[13, 14] have reported that adrenal cortical insufficiency, both in the rabbit and dog, results in no change in the concentration of serum complement. In the work reported here, there is no significant change in serum complement on the injection of cortical hormone into normal guinea pigs and into normal sensitized guinea pigs. It is probable, therefore, that adrenal cortical hormone does not influence anaphylaxis or immunity through an effect upon serum complement.

12038

Comparison of Growth of *Trichomonas foetus* and *Trichomonas vaginalis* in Chick Embryos.*

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Nelson[1] first reported the cultivation of *Trichomonas foetus* in

[14] Kapnick, I., and Cope, O., *Endocrinology*, 1940, **27**, 543.

* This study was made possible by Dr. E. D. Plass, Head of the Department of Obstetrics and Gynecology, College of Medicine, University of Iowa, and Dr. Chas. Murray, Dean of the Division of Veterinary Medicine, Iowa State College, to whom the authors express their sincere appreciation.

[1] Nelson, P. M., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 258.

chick embryos after inoculations of 1 cc of a culture into the allantoic sac. The eggs were opened 3 to 4 days after inoculation. Allantoic fluid from the most heavily infected embryos was used to carry the protozoan through 14 subcultures. Later, Levine, Brandly, and Graham,[2] following the same procedure, carried *T. foetus* through 23 passages and found that 12-day embryos were better than others in an 8- to 16-day series tested. Hogue[3] inoculated *T. foetus* into the allantoic sac of developing chick embryos and allowed the embryos to hatch or die. In one of 4 chicks that hatched, the protozoa were found in the yolk sac, esophagus, intestines, gall bladder, and cloaca 2 days after hatching. The other 3 gave negative results on fecal examinations. In embryos that died after inoculation, the trichomonads were also found in the digestive tract, yolk, and gall bladder.

The present report concerns a comparison of the growth of bacteria-free cultures of *Trichomonas foetus*[4] and *Trichomonas vaginalis*[5] in chick embryos. Also, successful subculturing of *T. vaginalis* in chick embryos is recorded for the first time.

After a few preliminary trials, *T. foetus* was established in chick embryos and was carried through 14 passages in 78 days. The inoculations were made into the allantoic sac as described below for *T. vaginalis*. The organisms seemed to grow better after the first few subcultures. Seven- to 14-day-old embryos were employed and were inoculated with 0.1 cc to 0.4 cc of trichomonas-containing material. The small inoculum may explain the slight difficulty encountered in establishing the infection. The eggs were opened from 4 to 7 days following inoculation. Observations and experiences were essentially the same as those already reported by others. Examination of the different egg parts showed that the protozoa developed in the allantoic fluid or the amnionic fluid, but were not found in the egg white, the yolk sac, the actual embryo or its blood stream.

The work with *Trichomonas vaginalis* was much more difficult. In initial trials with this organism, less than 0.5 cc of a heavy culture was injected into the allantoic sac. (Table I.) Irregular results led to trials with other methods of inoculation. Attempts to grow the trichomonads in an artificial air cell on the chorio-allantoic membrane met with total failure. Inoculations into the yolk gave

2 Levine, N. D., Brandly, C. A., and Graham, R., *Science*, 1939, **89**, 161.

3 Hogue, M. J., *Am. J. Hyg.*, 1939, **30** (Sec. C), 65.

4 Glaser, R. W., and Coria, H. A., *Am. J. Hyg.*, 1935, **22**, 221.

5 Trussell, R. E., *J. Iowa M. Soc.*, 1940, **30**, 66.

TABLE I.

Showing Number of "Takes" in Embryos When Injected with Small Numbers of *T. foetus* as Compared with the Number of "Takes" When Injected with Small and Large Numbers of *T. vaginalis*.

T. foetus		*T. vaginalis*			
Inoculations of 0.1 to 0.4 cc per egg		Inoculations of 0.1 to 0.4 cc per egg		Inoculations of 0.75 to 1.0 cc per egg	
No. eggs Protozoa present	No. eggs Protozoa not found	No. eggs Protozoa present	No. eggs Protozoa not found	No. eggs Protozoa present	No. eggs Protozoa not found
184	34	9	66	306	96

Note: After *T. foetus* was once established in chick embryos, it was readily carried in series through 12 subcultures in about 8 weeks, even though small inoculums were employed. On the other, *T. vaginalis* was not subcultured in embryos when a small inoculation was made, but was easily carried in series through 24 subcultures in 15 weeks when a large inoculum was used. The initial material for the larger inoculations had been grown through several subcultures in chick embryo allantoic fluid over liver infusion agar slants before it was employed.

negative results in preliminary trials although subsequent work with larger doses was more successful.

Since the allantoic sac inoculations appeared most promising, these were investigated further and 5- to 17-day-old embryos were tested in an attempt to find the time of greatest susceptibility. This appeared to be 6 days. In addition, the *T. vaginalis* was cultured in chick embryonic fluids over liver infusion agar slants (pH 6.8-7.0) in an effort to adapt the trichomonads to such an environment. An excellent growth occurred and the organism has now been carried for 7 months in this medium. After several weeks' growth, embryo inoculations were attempted with larger doses (0.75 to 1.0 cc) of the supernatant embryonic fluid culture. Six-day embryos were inoculated in the allantoic sac. A small hole was punched into the natural air cell and another hole was drilled through the shell over the embryo,avoiding the blood vessels. The needle was inserted about 5 to 8 mm into the latter opening and the injection completed after which the hole was sealed with paraffin. It is recognized that an occasional inoculation may have been made into the amnion. This is especially true in the younger embryos. After inoculation the developing embryos were incubated at a temperature of 35°C. Beginning 2 hours after inoculation the eggs were opened at intervals up to 10 days. No apparent multiplication occurred during the first 24 hours; in fact, it was often difficult to find trichomonads in hanging drops during that time. Maximal growth was noted after 2 to 4 days. Here again, the protozoa were found only in the allantoic or amnionic fluids.

Only eggs showing maximum multiplication of the protozoa

were used for subculture into new 6-day embryos. In the subcultures, an equal number of inoculated eggs were opened on the second, third, and fourth day after injection and within an hour the most heavily infected allantoic fluids were reinoculated into fresh embryos. Following this procedure, a series of 24 subcultures has been maintained. A few embryos died following inoculation, some because of bacterial contamination, some because of mechanical injury, and others from unknown causes. The protozoan continued to grow well for several days in dead embryos.

It appears that *T. vaginalis* is not particularly pathogenic for chick embryos although Hogue[6] found that *Trichomonas foetus* produced a substance that destroyed tissue culture cells.

In summary, it was found that *Trichomonas vaginalis* and *Trichomonas foetus* can be grown in chick embryos but that the former was more difficult to maintain and did not grow as profusely as *T. foetus*. These 2 protozoa are probably nonpathogenic under such conditions. *T. vaginalis* was successfully cultured in series in developing chicken embryos for the first time. There appears to be no advantage in maintaining a stock series of such cultures since the organisms can be maintained more readily in artificial media with much less danger of bacterial contamination and with a richer growth.

12039 P

Experimental Production of Dietary Liver Injury (Necrosis, Cirrhosis) in Rats.*

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Liver injury, mainly in the form of acute diffuse necrosis combined with fat infiltration, has occurred irregularly in young rats fed a diet devoid of vitamin B (casein 18%, sucrose 68, melted butter fat 8, cod liver oil 2, salt mixture 4) and supplemented with thiamine, ribo-

[6] Hogue, M. J., *Am. J. Hyg.*, 1938, **28**, 288.

* Independently and at the same time Dr. Graham T. Webster of the Department of Medicine, Western Reserve University, has made similar observations and has reached essentially identical conclusions. Publication of his results is pending.

flavin and pyridoxine.[1] In some of these livers there was diffuse periportal fibrosis. Rats fed the same basal diet plus yeast, or concentrated yeast extract, were free from any pathological changes in the liver. Later, the occurrence of cirrhosis of the liver on a nutritional basis and its prevention similarly by the addition of yeast to the diet were reported in rabbits[2] and in guinea pigs.[3]

The unpredictable and irregular incidence of liver injury in rats has been indirectly regarded as a basis for the assumption that the proper experimental conditions for the regular production of liver injury have not been provided by the experimental technic used.

In view of the well known lipotropic activity of casein,[4] the high proportion (18%) of casein in the basal diet was considered to be possibly one important factor which might counteract other conditions that would be conducive to liver injury. Consequently, rats weighing between 100 and 250 g were put on a diet that had the following composition: casein 10%, sucrose 64, lard 20, cod liver oil 2 and salt mixture 4. This modified basal diet was supplemented with thiamine, riboflavin, pyridoxine and pantothenic acid. In this group of rats the incidence of liver injury rose from an irregular occurrence, as it was in the rats on the original diet (with casein 18% and butter instead of lard), to a regular complication. Necrosis with or without cirrhosis and cirrhosis without necrosis were observed in rats that died between the 100th and 150th experimental days or were killed on the 150th day.

Addition of from 10 to 20 mg of choline daily reduced the incidence and the severity of the liver injury but not to a great extent. On the other hand, addition of from 10 to 50 mg of cystine daily proved to be the most potent factor in the accentuation of cirrhosis of the liver.

In this connection it should be noted that necrosis of the liver following high doses of cystine has been observed in the past.[5] In short term toxicological experiments even cirrhotic changes have been observed recently in rats which received excessive amounts of cystine (5 to 10% of the diet).[6]

1 György, P., and Goldblatt, H., *J. Exp. Med.*, 1939, **70**, 185.

2 Rich, A. R., and Hamilton, J. D., *Bull. Johns Hopkins Hosp.*, 1940, **66**, 185.

3 Spellberg, M. A., and Keeton, R. W., *Am. J. Med. Sc.*, 1940, **200**, 688.

4 *Cf.* Best, C. H., and Ridout, J. H., in Luck, J. M., Ed., *Annual Review of Biochemistry*, Stanford University, 1939, **8**, 349.

5 Curtis, A. C., and Newburgh, L. H., *Arch. Int. Med.*, 1927, **39**, 828; Sullivan, M. X., Hess, W. C., and Sebrell, W. H., *Pub. Health Rep.*, 1932, **47**, 75; Lillie, R. D., *Pub. Health Rep.*, 1932, **47**, 83.

6 Earle, D. P., and Victor, J., *J. Exp. Med.*, 1941, **73**, 161.

Daily addition of from 10 to 20 mg of choline or of 1 g of yeast or, better, of both, neutralized more or less completely the effect of cystine on the liver. The greater benefit achieved by choline in the presence of cystine over that seen in rats fed choline without additional cystine may be explained by the conception that choline acts through the intermediary of cystine.

The pathogenesis of dietary liver injury (necrosis, cirrhosis) presented here is closely connected with the lipotropic effect[4] of casein and with fat infiltration of the liver which has been considered a prerequisite of cirrhosis.[7]

The results presented here are based on experiments made on a total of 264 rats. The work is being continued.

12040

Effect of Parenteral Administration of Vitamin B_1 and Vitamin B_6 on a Coccidium Infection.*

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From Iowa State College.

In a paper now in press[1] it has been shown that the addition of both vitamins B_1 and B_6 to a special ration generally brought about a very striking reduction in the number of oöcysts produced in rats infected with standardized doses of the coccidium *Eimeria nieschulzi*. Since vitamin B_1 supplement alone only somewhat reduced the number of oöcysts eliminated and vitamin B_6 supplement increased the counts, the striking reduction brought about by combination of the two was entirely unexpected. In order to determine whether the synergistic action of the vitamins was dependent upon their admission to the site of the infection through the intestinal route, a series of tests has been carried out in which the two vitamins were administered intraperitoneally in normal saline solution instead of being mixed with the ration. Otherwise the procedure and technics were in general the same as previously described.[1]

The animals in lots 1 and 2 averaged about 70 g when started on the rations; those in lot 2, 120 g; lot 3, 90 g. The test and the reference

[7] Connor, C. L., *Am. J. Path.*, 1938, **14**, 347; *J. A. M. A.*, 1939, **112**, 387; Chaikoff, I. L., and Connor, C. L., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 638.

* Aided by a grant from the American Academy of Arts and Sciences.

[1] Becker, E. R., and Dilworth, R. I., *J. Inf. Dis.*, 1941, in press.

TABLE I.
Mean 16-day Weight Gains and Mean Number of Oöcysts Eliminated by Rats Infected with *Eimeria nieschulzi*.

	Test Series				Reference Series			
			No. oöcysts				No. oöcysts	
Lot	No. rats	Wt gains, g	Millions	S.E.	No. rats	Wt gains, g	Millions	S.E.
1	5	50	22.0	± 8.2	5	28	208.2	±27.7
2	9	37	17.1	± 8.4	8	26	150.8	±17.4
3	7	43	61.4	±21.7	6	25	210.8	±13.2
4	8	42	94.1	±30.2	8	24	127.1	±17.0

series were in each case composed by dividing litters of similar ages and not previously infected, as nearly equally as possible according to weight and age. The reference ration was made up in parts by weight as follows: beet-sugar, 69; casein (unextracted), 14; soy bean oil meal, 6; normal salts (Harris), 4; fine cellulose, 2; lard, 3; cod liver oil, 2. The test series was also fed this reference ration, but in this case each animal received every other day into the body cavity an injection of 15 μg vitamin B_1 and 40 μg vitamin B_6. In the case of lots 1 and 2, the reference rats received an injection of 40 μg vitamin B_6 every other day, but no B_1. In lots 3 and 4 the reference rats were not injected. The weight gains appearing in the table represent the mean maximum for the series attained during the first 16 days of the experiments, and the number of oöcysts the mean number passed by the series during the entire course of an immunizing infection.

The results obtained were no less striking than when the vitamins were fed. In lots 1 and 2 (Table I) the reference series produced practically 9 times as many terminal stages of the parasite (oöcysts) during the infection as the test series; in lot 3, between 3 and 4 times as many. When the differences between the counts in each of the first 3 lots were tested for significance by Fisher's small sample method, the values of P obtained indicated high significance. Lot 4 deserves special comment, for in this case there were 2 counts in the test series that far exceeded even the highest in the reference series, so that a mean of 94.1 was obtained, even though the remaining 6 counts averaged but 46. A few similar cases were encountered in the previously mentioned experiments[1] in which the vitamins were fed.

Conclusion. Vitamins B_1 and B_6 have an inhibiting influence on the development of *Eimeria nieschulzi* infections in rats on a special ration when administered by a parenteral route, one at least as striking as when the vitamins are fed.

12041

Effect of Oxalic Acid Given Intravenously on the Coagulation Time in Hemophilia.

JOHN B. JOHNSON.* (Introduced by W. S. McCann.)

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Steinberg and Brown[1] reported the preparation of extracts from certain plants which they stated, caused a rapid acceleration of the rate of coagulation of blood. The active agent in this extract was identified as oxalic acid. They stated that elevation in the clotting time was attended by a fall in blood oxalic acid, while reduction in clotting time conversely resulted in a rise in the oxalic acid content of the blood. The extract was marketed under the name Koagamin.

Steinberg[2] indicated that this extract was effective in hemophilia. Schumann,[3] reporting on the use of Koagamin in the control of hemorrhage, stated that Koagamin had been used in hemophilia with gratifying results. However, he gives no data nor references to illustrate that fact. Page and coworkers[4] recently reported 3 hemophiliacs in whom the coagulation time was said to become normal after the injection of oxalic acid intravenously. They stated that one of the cases became refractory to oxalic acid injections after the first course of therapy. They used 3 to 5 cc of a 0.1% solution of oxalic acid 3 times daily. These results, if confirmed, would represent a tremendous advance in the clinical management of hemophilia.

This communication reports the use of oxalic acid in 5 cases of hemophilia. Two of the cases were also treated with Koagamin. Our results indicate that neither Koagamin nor oxalic acid causes any reduction in the coagulation time in hemophilia.

Method of Study. Five hemophiliacs from 4 different families were used in the study. These patients all have typical family histories and clinical findings of the disease. The coagulation time was measured by the following procedure: The coagulation tubes were 8 mm in diameter. They had been cleaned with bichromate-

* Rockefeller Foundation Fellow in Medicine.

1 Steinberg, Arthur, and Brown, William R., *Am. J. Physiol.*, 1939, **126**, 638.

2 Personal communication.

3 Shumann, Edward A., *Am. J. Ob. and Gyn.*, 1939, **38**, 1002.

4 Page, R. C., Russell, H. K., and Rosenthal, R. L., *Ann. Int. Med.*, 1940, **14**, 78.

sulphuric acid solution, rinsed with distilled water and, just before using, were again rinsed with physiological saline. Twenty-five cc syringes and 20 gauge needles, both rinsed with physiological saline, were used for venapuncture. The sample was not used if the puncture was not immediately successful. At least 10 cc of blood were drawn for each determination. One cc of blood was run into each of 4 of the coagulation tubes, which were placed in a water bath at 28°C. The tubes were tilted at approximately 3-minute intervals, until they could be inverted without loss of contents. The average time of the 4 tubes was used as the coagulation time of the sample. The normal coagulation time by this method is 6 to 12 minutes.

The oxalic acid solution contained 1 mg of oxalic acid C.P. per cc of distilled water. The solution was sterilized by autoclaving at 15lb pressure for 20 minutes. The Koagamin and Koaginol were obtained from Dr. Steinberg and given according to his directions.

Report of Cases. Table I shows the effect of oxalic acid on the coagulation time of 3 of the 5 cases studied. The dosage varied from 10 to 27 mg daily given intravenously. No reduction in the coagulation time was obtained in any of these 3 cases.

Figs. 1 and 2 are graphic representations of the ineffectiveness of the oxalic acid therapy. The average daily dose in Case 5 (Fig. 2) was 47 mg. Case 4 developed a hemorrhage in the forearm on the first day of oxalic acid therapy which progressed under continued oxalic acid administration. Figs. 1 and 2 also show that these patients are typical in their response to agents which are usually effective in reducing the coagulation time in hemophilia. Attention is called to Fig. 2, which shows the effectiveness of lyophiled human plasma in reducing the coagulation time in hemophilia. Details of this form of therapy will be given in a later publication.

Table II shows the failure of Koagamin and Koaginol to reduce the coagulation time in Case 5. Similarly, no reduction in the coagulation time was obtained in Case 1 after the intravenous injection of Koagamin.

TABLE I.

Effect of Oxalic Acid Intravenously on the Coagulation Time in Hemophilia.

Case No.	Oxalic acid mg per day I.V.	Duration of treatment days	Coagulation time in minutes					Comments
				Days after treatment				
			Control	1	2	3	4	
1	10	5	67	57	63	64	64	
2	12	3	67	75		65		Bled for 3 days from vena puncture third day.
3	27	3	86	81	84	82		

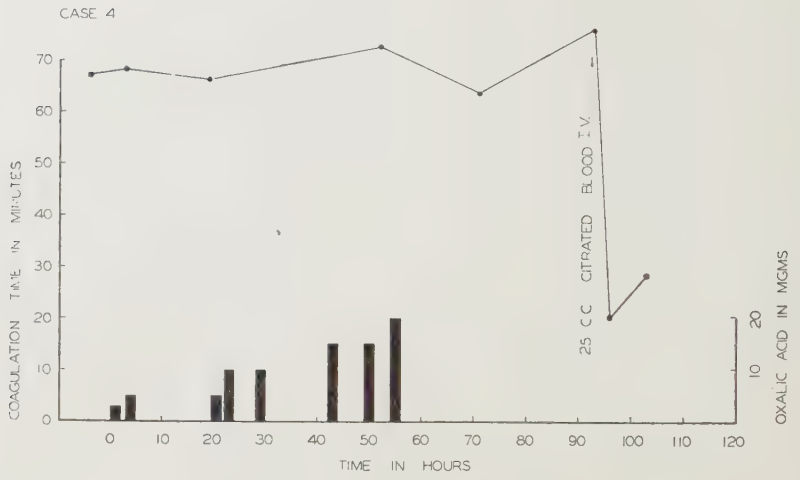


FIGURE I EFFECTS OF OXALIC ACID INTRAVENOUSLY ON COAGULATION TIME IN HEMOPHILIA

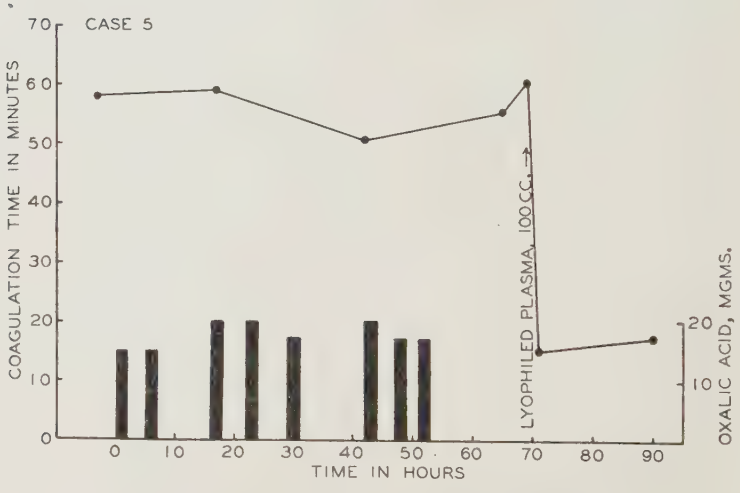


FIGURE II. EFFECT OF OXALIC ACID INTRAVENOUSLY ON COAGULATION TIME IN HEMOPHILIA

Discussion. Our results indicate that oxalic acid in doses from 10 to 47 mg daily by vein, causes no reduction in the coagulation time in hemophilia. Patients from 4 different families have been studied in order to avoid any unusual type of response peculiar to a given family.

We are unable to explain the negative results in our cases as compared with those of Page, *et al.* The fact that they drew only one cc of blood for each test may be one of the factors. We have

TABLE II.
Effect of Koagamin and Koaginol on Coagulation Time in Hemophilia.
Case 5.

Date	Time	Medication	Coag. time, min
9/27		Control	85
9/28		"	88
9/29	8:40 A.M.	Koagamin { 3 cc I.V. Koagamin { 2 " I.M.	
9/29	9:40 "	" 2 " I.M.	99
9/29	10:40 "	" 2 " I.M.	
9/29	11:40 "	" 2 " I.M.	
9/29	2:40 P.M.		97
9/30	2:10 "	" 5 " I.V.	
9/30	4:35 "		100
9/30	4:38 "	Koaginol 2 " I.M.	
10/1*	10:00 A.M.		106

*Large hematoma at site of Koaginol injection.

found this an unreliable procedure as small amounts of tissue juice will materially change the coagulation time when only one cc of blood is withdrawn. We have no explanation for the negative results with the use of Koagamin and Koaginol.

Conclusion. The intravenous injection of Koagamin and oxalic acid in the doses used in this study had no effect on the coagulation time in 5 cases of hemophilia.

Grateful appreciation is expressed to Dr. John Lawrence for criticisms and suggestions.

APPENDIX.

Case 1. Unit No. 27018. Male. Age 41. Family history of hemophilia. One brother with hemophilia. Has had numerous episodes of hematuria and joint hemorrhages. Has several ankylosed joints. Blood calcium, fibrinogen, and prothrombin time, normal.

Case 2. Unit No. 86680. Male. Age 12. One of 3 hemophiliacs of present generation. Family history of hemophilia. Has joint hemorrhages (repeated) and many prolonged episodes of bleeding from minor injuries.

Case 3. Unit No. 27899. Male. Age 43. History similar to that of brother, Case 1. Hemorrhagic episodes dating from infancy through present time.

Case 4. Unit No. 9987. Male. Age 20. Family history of hemophilia. Has had repeated joint hemorrhages. First episode of hemorrhage at 9 months. Excessive bleeding from minor injuries since infancy. Blood calcium fibrinogen normal.

Case 5. Unit No. 77669. Male. Age 26. Family history of hemophilia. First hemorrhages at 3 weeks of age. Later severe hemorrhage after circumcision. Repeated episodes of hemorrhage into G.I. tract, joints, and muscles. Several episodes of hematuria, epistaxis, and laryngeal hemorrhage. Blood calcium, fibrinogen, and prothrombin time, normal.

12042 P

A Comparison of Antigens by Interfacial and Nephelometric Methods.

RALPH J. DEFALCO. (Introduced by T. C. Nelson.)

From the Zoological Laboratory, Rutgers University.

The results obtained by Boyden[1] with the Libby photronreflectometer[2] indicate clearly the value of this instrument in studies of precipitin. The instrument affords an accurate measurement of the precipitate formed by antigen and antibody. Light rays passing through the turbid system are reflected by the suspended particles, and falling upon a photronic cell generate a current of electricity which is recorded by a galvanometer. The galvanometric readings, obtained with doubling dilutions of antigen and a constant amount of antiserum, furnish the points for a curve, the area under which is used to indicate the total reaction between the antigen and antiserum. A comparison of the areas of the homologous and heterologous reaction-curves is used herein to indicate the chemical similarity. In the interfacial tests, relationship-values were determined in the manner described by Boyden,[3] the end-point being the all-important factor.

The graphs show the results obtained when several antigens were tested with a diluted (1+2) anti-leghorn serum. Each antigen represents the pooled sera of from 4 to 95 birds, with the exception of turkey-buzzard which is the serum of one individual. In a similar

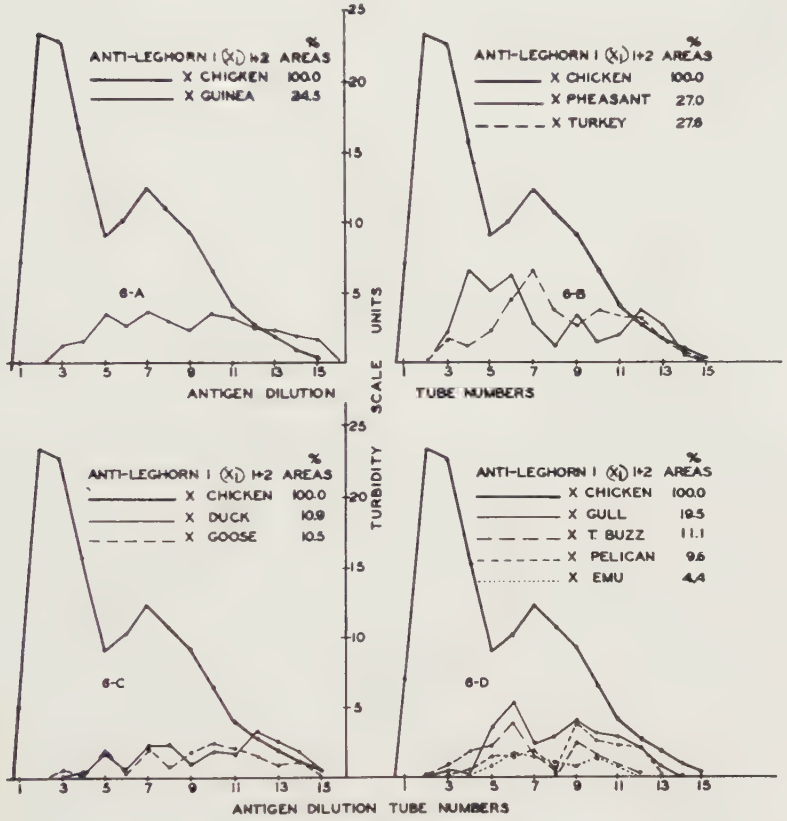
TABLE I.
A Comparison of Interfacial (I) and Nephelometric Values (N) Using the Same Antiserum for Both Tests.

Antigens	Anti-leghorn serum (X_1)		Anti-guinea hen serum (X_1)		Anti-turkey serum (X_1)		Anti-duck serum (X_3)		Anti-pelican serum (X_2)	
	I	N	I	N	I	N	I	N	I	N
Leghorn	100.0	100.0	25.0	32.5	25.0	53.2	0.8	28.7	0.8	30.9
Guinea hen	1.6	24.5	100.0	100.0	25.0	18.2	0.8	—	1.6	19.7
Turkey	50.0	27.6	12.5	22.0	100.0	100.0	0.8	—	0.8	—
Pheasant	50.0	27.0	50.0	34.7	12.2	37.8	0.4	20.7	0.4	—
Goose	3.1	10.5	25.0	9.8	6.3	7.2	50.0	70.0	0.8	—
Duck	3.1	10.9	12.5	6.8	3.1	—	100.0	100.0	3.1	3.7
Turkey buzzard	3.1	11.1	12.5	5.1	0.4	7.8	6.3	—	6.3	18.3
Pelican	3.1	9.6	12.5	2.4	0.0	13.6	1.6	10.3	100.0	100.0
Gull	0.0	19.5	25.0	5.8	1.6	—	0.4	—	1.6	26.5
Emu	0.4	4.4	12.5	0.1	0.0	—	0.4	—	1.6	—

[1] Boyden, A. A., *Carnegie Inst. Wash. Year Book*, 1938-1939, 219.

[2] Libby, R. L., *J. Immunol.*, 1938, **34**, 71.

[3] Boyden, A. A., and Noble, G. K., *American Museum Noviates*, 1933, No. 606.



manner the same antigens were tested with the precipitating antisera to the serum-proteins of turkey, guinea hen, duck, and pelican. The table shows the values obtained by the interfacial (ring) and nephelometric methods.

The injection of a mixture of antigens, such as serum, may result in the production of: (a) antibodies to one fraction alone; (b) antibodies to several or all fractions, with more to one fraction than to another. The fractions are rarely present in equal amounts, so the interfacial endpoints may, in some cases, be the result of one fraction and its antibodies, and in other cases the result of another fraction with its antibodies. Since in relationship-studies the antiserum is kept constant, a small but strongly antigenic fraction spends the greater part of its reaction within the first few tubes, whereas, a larger but less antigenic fraction yields a wider prezone and positive reactions occur in the higher dilutions of antigen. Thus, while the interfacial test is a good test of the sensitivity of the antibodies, it measures only one point at the end of a curve. The

nephelometric test is a better indication of the similarity of the antigenic substances, for it registers the total reaction, involving all antigens and all antibodies in the reacting systems.

12043

An Attempt to Produce Hepatic Cirrhosis by a Diet Deficient in Vitamin B Complex.

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Interest in the possible rôle of a vitamin deficiency in the production of cirrhosis of the liver has resulted from (1) the clinical observation that patients with that disease have improved following the administration of a high vitamin diet (Patek[1]), and from (2) the experimental observations that hepatic necrosis developed in rats offered a vitamin deficient diet (György and Goldblatt[2]) and more recently that cirrhosis without necrosis occurred in rabbits on a diet deficient in some factor that is contained in yeast (Rich and Hamilton[3]). The latter investigators believed that a lack of thiamin, riboflavin, or nicotinic acid could be eliminated as the responsible factor. In order to investigate this matter in another species of animal we have administered to the rat one of the diets that Rich and Hamilton found effective for the production of hepatic cirrhosis, but have failed in that animal to secure comparable results.

Procedure. Eighteen apparently normal, male, adult, albino rats of an inbred Wistar strain were selected. The mean body weight was 300 ± 80 g. Twelve of these animals were placed on a diet deficient in the vitamin B complex, while the remaining 6 received B complex in the form of brewers' yeast and served as controls. All animals in groups of three received daily a food bolus consisting of

* Arthur W. Thompson Fellow in Gastroenterology.

† Student Volunteer Research assistant.

1 Patek, A. J., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 329.

2 György, P., and Goldblatt, H., *J. Exp. Med.*, 1939, **70**, 185.

3 Rich, A. R., and Hamilton, J. D., *Bull. J. H. Hosp.*, 1940, **66**, 185.

10 g of vitamin-free casein obtained from the Borden Co.; 4 g of "Crisco"; 25 g of dextrin (Baker's N F V white powder); 24 g of boiled rice; 25 g of pulpified raw carrots, and 4 g of salt mixture (Weech, *et al.*[4]). The six control animals received powdered brewers' yeast (Harris) mixed with the bolus of food (5 g of yeast per 3 rats). The weighed amount of food placed in the cage daily was in excess of that eaten; the uneaten portion was weighed and discarded. Each animal was given in addition, every second day by means of a stomach tube, vitamins A, D, and E (0.23 cc of a mixture of 4.4 cc of peanut oil, which contained 50 mg of Merck's alpha tocopherol and 29.2 cc of U.S.P. cod liver oil).

The rats, in groups of 3, were housed in cages, the bottoms of which were made of large-mesh wire screening, so as to prevent refection. The cages were also so constructed and arranged as to prevent the access of food or feces to the animals of another cage. A measured amount of fresh water was supplied daily and the 24-hour intake measured. Body weight was recorded daily.

The 12 animals on the B-complex deficient diet were allowed to live until death occurred spontaneously, except in 3 instances in which it was evident that demise would occur during the next few hours; these were decapitated under light ether anesthesia so as to obtain specimens that would be free of changes due to postmortem autolysis. Four animals were subjected to exploratory laparotomy on the 36th day of the experiment. At death the liver of each rat was removed, weighed and fixed in 10% formalin solution. Sections were stained with hematoxylin eosin, Masson's trichrome connective tissue stain and scarlet red.

Results. The 12 animals on the deficient diet maintained their weight for one week, and then showed a progressive loss that continued until their demise, which occurred, on the average, when 51.2% of the initial body weight had been lost. The control animals gained weight steadily during the first 8 weeks of the experiment, and then the weight tended to remain fairly constant (Fig. 1).

The average food intake of the individual rat on the deficient diet for each of the first 5 days of the experiment was 27 g. This decreased progressively to 10 g during the twelfth week. The controls, in contrast, ingested an average of about 30 g per day during the first 5 days; this decreased to about 26 g by the end of the second week and then remained at that level throughout the rest of the experiment. The daily consumption of water paralleled rather closely that of the food in both groups of animals, decreasing in the

[4] Weech, A. A., Goettsch, E., and Reeves, E. B., *J. Exp. Med.*, 1935, **61**, 299.

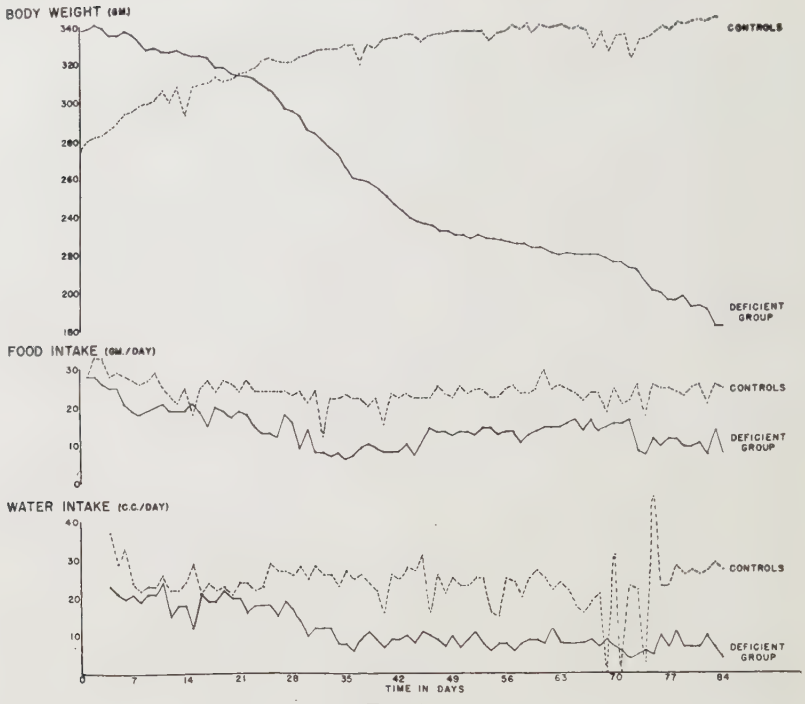

FIG. 1.

The trend in body weight, food and water intake of animals on a B-complex deficient diet (solid lines) and of control animals (dotted lines) receiving brewers' yeast over a period of 12 weeks. Each point on the curves represents the average per animal in a group of 3 per cage. The fluctuations in water intake of the control animals from the 70th to the 77th day are artefacts due to clogging of the glass tubing of the inverted water bottle by air bubbles.

deficient group and being more or less constant in the controls (Fig. 1).

Along with the progressive loss of weight in the deficient group, there occurred a looseness of the skin and a shagginess of the coat of hair, some of which finally fell out in patches, particularly over the haunches. A low grade dermatitis of the margins of the ears and of the inner aspects of the paws and feet appeared. During the terminal stages, the animals had a characteristic "hunched up" appearance. The wasting of the subcutaneous tissues resulted in a prominence of the osseous structures, and the animals appeared to hold their balance with difficulty. Tetanic convulsive movements were frequently observed just prior to demise. No bleeding from the skin or mucous surfaces and no keratinization of the eyelids occurred. (Fig. 2.) The eyegrounds retained their pink color throughout the experiment. The 6 controls had sleek coats and otherwise appeared to be in excellent physical condition. (Fig. 2.)

Fig. 2.

Photograph of animals after 9 weeks on the diet. The animal in the center received the basic diet plus brewers' yeast (control), while the others did not receive the brewers' yeast. The animal on the right is practically moribund as a result of advanced B-complex deficiency while the one on the left is as yet but moderately deficient.

The 4 animals on the deficient diet that were subjected to exploratory laparotomy after 5 weeks, with the intention of removing some hepatic tissue for biopsy, had in each instance a normally appearing liver which made the latter procedure unnecessary. All of the animals on the B-complex deficient diet lived at least 6 weeks except one that died during the 6th week. Two lived 13 weeks, and the remainder either were killed or died within that length of time. Thus most of the animals lived sufficiently long for cirrhosis to develop, were it going to.

Pathologic Findings. All the livers of the animals on the B-complex deficient diet were decreased in size, having an average weight of 4.56 g as compared to an average of 10 g for the controls. The surface of the organ was smooth, glistening and deep red in color. No exaggeration of lobular markings or irregularity of the surface was observed. The livers of the control animals were large, the surfaces smooth and the color light red, suggesting an increased amount of fat.

Microscopically, the sections of the livers of the vitamin-deficient animals showed no evidence of degeneration of the hepatic cells or of proliferation or condensation of connective tissue. The cells were small and did not contain stainable fat. The hepatic cells of the control animals, on the other hand, were large and contained stainable fat in small droplets scattered throughout. These also showed no evidence of hepatic cell necrosis, fibrous tissue proliferation or condensation.

Comment. Rats administered the identical diet on which rabbits have been reported to have developed hepatic cirrhosis failed to do so, though some of the animals took the diet for as long as 13 weeks.

They did, however, exhibit recognized manifestations of B-complex deficiency, suggesting the probability of some other factor being responsible for the hepatic cirrhosis observed in rabbits by Rich and Hamilton or for its absence in our rats.

One possibility was that the carrots and rice, or possibly the peanut oil, contained enough of the unknown cirrhosis-preventing factor to interfere with the development of the disease in rats. Accordingly, another group of 6 rats (body weight, 198 ± 30 g) was placed on a diet similar to the one described except that the boiled rice and raw carrots, also alpha tocopherol in peanut oil, and cod liver oil, were omitted. These animals, younger than those previously used, also failed to develop hepatic lesions. They lost weight rapidly and were dead within 35 days of the onset of the experiment. In this connection it is to be noted that one of the rabbits described by Rich and Hamilton was observed to have cirrhosis after 25 days on the diet.

Another possible explanation of the negative observations in the rat under conditions similar to those reported to produce hepatic cirrhosis in the rabbit may be a species difference; the rat may be less susceptible. György and Goldblatt reported observation of focal or patchy hepatic necrosis in some of a group of rats kept on a vitamin B-complex deficient diet. The lesions, however, could not be produced at will, and they did not occur regularly under identical conditions. The apparently chance occurrence of the lesions raises the question whether they were the result of the experimental procedure instituted or were related to coincidental factors. A case in point is that in which hepatic necrosis in rats was described by Davis, *et al.*,[5] following the administration of sulfanilamide. Similar lesions were found by Machella and Higgins[6] in the livers of rats receiving sulfanilamide, and also in apparently normal animals serving as controls and not receiving the drug. Such lesions have been ascribed by Buchbinder, *et al.*,[7] to an enzootic form of paratyphoid infestation.

In this same category lies the question whether the cirrhotic process in the liver of rabbits noted by Rich and Hamilton was due to infestation with coccidia. Ophuls[8] and also Smetana[9] have

[5] Davis, H. A., Harris, L. C., Jr., and Schmeisser, H. C., *Arch. Path.*, 1938, **25**, 750.

[6] Machella, T. E., and Higgins, G. M., in press.

[7] Buchbinder, L., Hall, L., Wilens, S. L., and Slanetz, C. A., *Am. J. Hygiene*, 1935, **22**, 199.

[8] Ophuls, W., PROC. SOC. EXP. BIOL. AND MED., 1910, **8**, 75.

[9] Smetana, H., *Arch. Path.*, 1933, **15**, 516.

demonstrated that this organism is capable of inciting fibrous tissue proliferation in the perilobular zones in the livers of rabbits. In this connection, furthermore, it is noteworthy that the animals used by Rich and Hamilton were infested with coccidia, and that one of their control animals, which took the diet containing brewers' yeast, but poorly, developed cirrhosis. In agreement with the above is the recent work of Spellberg and Keeton,[10] who reported the development of hepatic cirrhosis in a rabbit on a seemingly adequate diet which contained B-complex as found in cereal foods, and also in a guinea pig on a somewhat similar diet but supplemented with desiccated brewers' yeast.

Summary. Adult male albino rats administered a vitamin B-complex deficient diet over a period of from 6 to 13 weeks failed to develop histologic evidence of hepatic cirrhosis, in spite of the fact that they did develop the usual signs of B-complex deficiency. Data as to food and water intake as well as weight changes are presented.

12044

Effect of Pantothenic Acid Alone and in Natural Products on Nutritional Achromotrichia in Rats.

D. V. Frost, Ruth C. Moore and F. Peirce Dann. (Introduced by C. Nielsen.)

From the Abbott Laboratories, North Chicago, Illinois.

Within the last year much attention has been centered around the rôle of pantothenic acid[1-4] in prevention and cure of nutritional achromotrichia. That pantothenic acid can be identified *in toto* with the anti-graying potency of natural products, such as rice bran, liver and yeast, has been claimed by certain workers[1, 3] and denied by others.[2, 4, 5] Recently György[6] has named biotin (vitamin H) as an additional factor in normal pigmentation, but the evidence presented was not entirely conclusive. Results of work in our laboratory

10 Spellberg, M. A., and Keeton, R. W., *Am. J. Med. Sc.*, 1940, **200**, 688.

1 György, P., Poling, C. E., and Subbarow, Y., *J. Biol. Chem.*, 1940, **132**, 789.

2 Dimick, M. K., and Lepp, A., *J. Nutrition*, 1940, **20**, 413.

3 Unna, K., and Sampson, W. L., Proc. Soc. Exp. Biol. and Med., 1940, **45**, 309.

4 Williams, R. R., *Science*, 1940, **92**, 561.

5 Nielsen, E., Oleson, J. J., and Elvehjem, C. A., *J. Biol. Chem.*, 1940, **133**, 167.

6 György, P., and Poling, C. E., Proc. Soc. Exp. Biol. and Med., 1940, **45**, 773.

indicate that pantothenic acid is *not* the only factor involved. What other factor, or factors, is involved remains an interesting question.*

Careful analysis of various natural products for pantothenic acid was essential to the interpretation of their anti-graying effects and for the clear postulation of the involvement of other unknown factors. The chick pantothenic acid assay method used was a modification of the Jukes method[7] in which the whey adsorbate component of the diet was replaced by the crystalline B-complex factors. When the typical dermatitis syndrome with incrustations around mouth and eyes developed after one week on a complete diet and 2 weeks on the deficient diet, the chicks were placed on test. A solution of d-calcium pantothenate served as a standard for each group with 7 chicks per dose level. Supplements were administered by pipette and cure of dermatitis rather than growth was used as the criterion of the activity of test materials. The chick assay method was checked in all cases by a quantitative microbiological assay using lactobacillus casei.† Correlations between the two methods were generally good, although the chick method yielded much higher results with certain products, notably brewer's yeast. This discrepancy cannot be explained satisfactorily at present.

Experiments (Table I) were designed to determine the anti-graying potency of liver and yeast extracts and to correlate this potency with the pantothenic acid content. In these experiments graying was produced in from 5 to 10 weeks in young rats on the following ration: Casein (Labco) 18; sucrose, 73; salts O-M, 4; corn oil, 3; cod liver oil, 2. The B-complex factors were added to the ration in amounts calculated to provide the following amounts daily: thiamin, 20 μg; riboflavin, 40 μg; pyridoxine, 20 μg; nicotinic acid, 120 μg, and choline, 5 mg. Supplements were fed for 7 weeks, and the effect on pigmentation carefully noted. In all cases the liver extracts were more effective than their equivalence of pure synthetic d-calcium pantothenate. Although, in some instances, the anti-graying potency of extracts paralleled their pantothenic acid content, certain samples, notably liver fraction and brewer's yeast, had much higher anti-graying potency than the relative pantothenic acid content warranted. The animals which developed the gray hair syndrome in 35 days did not cure any more rapidly than those which had taken 60 to 70 days to deplete.

* Ansbacher, S. (*Science*, 1941, **93**, 164), named p-aminobenzoic acid as a chromotrichia factor for rats after this paper had been prepared.

7 Jukes, T. H., *J. Biol. Chem.*, 1937, **117**, 11.

† Method of Prof. F. M. Strong conducted by Eleanor Roedger.

A butanol extract of autolyzed liver proved to be an excellent source of the anti-graying factors supplementary to pantothenic acid. This fraction fed at a level calculated to supply only 15 μg of pantothenic acid per day failed to cure graying. When 35 μg of synthetic d-calcium pantothenate was added to this therapy, the graying was clearly alleviated though not completely cured in all rats.

Synthetic d-calcium pantothenate (200 μg) was injected daily for a period of 7 weeks into 3 piebald rats after graying had developed. At the end of this period there appeared to be no difference between the injected rats and the controls, which had been fed an equal amount of calcium pantothenate. All of the rats were about equally gray at the end of the experimental period and had gained weight constantly throughout the experiment.

Concurrently with the fore-mentioned work we attempted to determine the effect of varying levels of d-calcium pantothenate on growth and incidence of graying in young black or piebald rats on a highly purified diet. The composition of the diet was as follows: Casein (Labco-refluxed and washed with 95% 3A ethyl alcohol), 74; corn oil, 3; salts, 4; haliver oil, 1; thiamin, 0.4 mg; riboflavin,

TABLE I.
Pantothenic Acid of Natural Products Versus Synthetic d-Calcium Pantothenate in the Cure of Achromotrichia.

Sample	Daily dose, mg	Pantothenic acid in daily dose, μg: Chick assay	Pantothenic acid in daily dose, μg: Microbiol. assay	Curative effect on gray hair syndrome*
Liver Paste X	50	25	28	1P, 3N
" " X	100	50	55	4C
" " W	100	45	53	2C, 3P
" " A	100	40	49	2C, 2P, 1N
" " A	150	60	74	1C, 2P, 1N
" Powder W	100	60	34	3P, 1N
" " W	150	90	51	4C
" " Y	150	56	25	2C, 2P
" " Y	200	75	33	1C, 2P, 1N
95% Alcohol Sol. Liver Paste	100	50	46	4C, 1P
Brewers' Yeast Powder	250	30	4	4P, 1N
" " "	500	60	8	4C
Ca Pantothenate	.025			1P, 3N
" "	.050			2P, 3N
" "	.075			5N
" "	.100			1C, 4P
" "	.200†			1P, 2N
" "	.200‡			2P, 1N

*C—Complete cure. P—Partial cure. N—No cure.
†Oral administration.
‡Intramuscular administration.

0.4 mg; pyridoxine, 0.4 mg; nicotinamide, 20 mg; and choline, 100 mg. Black and piebald rats 21 days old were divided into 4 groups of 6 each, with 3 males and 3 females in each group. The amounts of d-calcium pantothenate supplied daily by mouth were: Group I, 15 μg; Group II, 30 μg; Group III, 60 μg; and Group IV, 120 μg. Composite growth curves for the 6 rats in each group are shown in Fig. 1. There appeared to be no difference in the susceptibility of any of these groups to graying, although the degree of adequacy of pantothenic acid is clearly reflected in the gradation of the growth curves. Several of the rats were finally fed higher levels of d-calcium pantothenate, *i. e.*, up to 200 μg per day, without apparent benefit. Unna[8] has suggested 80 μg of d-calcium pantothenate per day as the minimum requirement for optimal growth and this is in line with our findings.

Contrary to the reports of Unna[9] and Jukes[10] we did not observe successful matings on the last mentioned highly synthetic diet with added calcium pantothenate. In each case the female apparently destroyed the male within 4 days after they were caged together. Prolonged bleeding from small paw injuries was noted in many

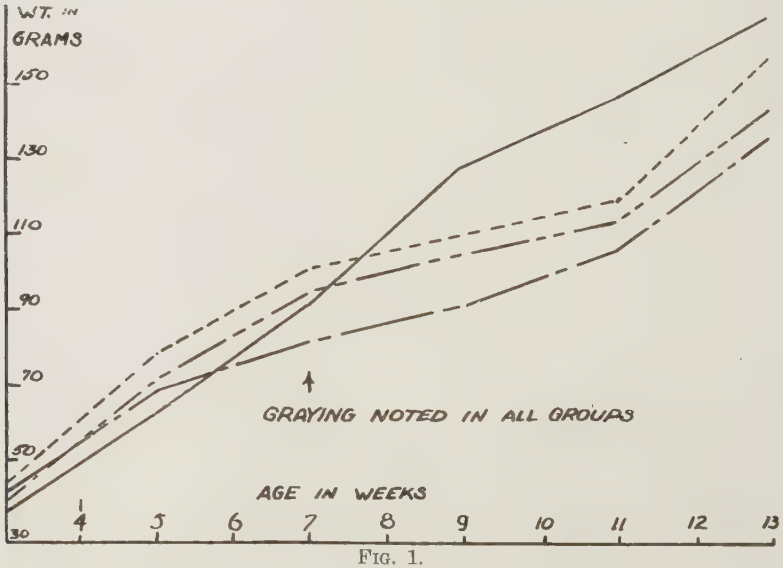

FIG. 1.

Effect of graded daily doses of synthetic d-calcium pantothenate on rat growth and complete inefficacy to prevent graying.

8 Unna, K., *J. Nutrition*, 1940, **20**, 565.

9 Unna, K., *Am. J. Med. Sci.*, 1940, **200**, 848.

10 Jukes, T. H., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 625.

cases. Jukes[10] noted a like condition in rats on similar rations and suggested that a vitamin K deficiency might exist.

Conclusion. The effect of pantothenic acid in cure of nutritional achromotrichia is difficult to assess at present because other factors are clearly involved. Certain liver extracts providing as low as 40 μg of pantothenic acid per day caused complete remission, while the effect of synthetic d-calcium pantothenate was negligible at best. The addition of d-calcium pantothenate to extracts low in this factor, however, increased their anti-graying potency. Age or weight increase of animals did not seem to influence the curative effect of supplements for graying.

Although rats were raised to maturity on highly synthetic diets supplying adequate d-calcium pantothenate, successful matings were not observed.

12045 P

Hyperplasia and Hypertrophy of the Mucosa of Larger Biliary Ducts in Mice Receiving Estrogens.*

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During the course of observations on mice which had received injections of various estrogens for prolonged periods an enlargement of the bile ducts was observed in many animals. In untreated mice the bile ducts, cystic duct, common duct and its branches, were small thread-like structures. Occasionally a slight cystic distension of the common bile duct was apparent at autopsy. The walls were delicate and when slightly distended almost transparent. Histologically the ducts had a thin and irregular muscularis, and a well-developed mucosa. The tall columnar epithelium was moderately folded on the loose underlying tissue of the lamina propria, and a few small glands extended to the serosa, especially in the common bile duct (Fig. 3).

The bile ducts of many mice which had received estrogens were grossly thickened to several times the size of the ducts of the untreated (Fig. 1). They are also rather rigid, white and somewhat

* This investigation has been supported by the Anna Fuller Fund and the Jane Coffin Childs Memorial Fund for Medical Research. The mice used were from the colony of Dr. L. C. Strong.

nodular. Enlargement was usually greatest at the points of junction with the branches leading to the several lobes of the liver. The main duct tended to become smaller as it approached the gut. The cystic duct was usually thickened up to the neck of the gall bladder. The gall bladder was involved in only a few mice and in these it was reduced in size and histologically resembled the upper end of the cystic duct.

Microscopic examination of the enlarged ducts of the estrogen-

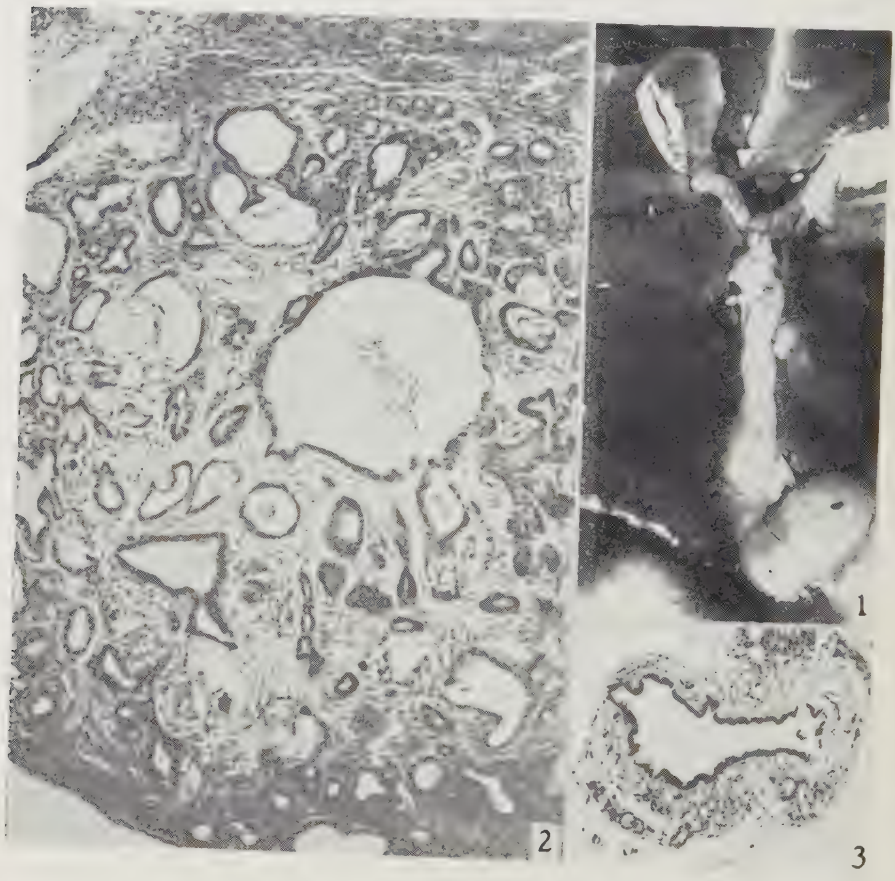

FIG. 1.

1. Gall bladder, cystic and common bile ducts of a F_1 hybrid mouse (A X C_3H) which had received 16.6 μg estradiol benzoate weekly for 247 days. The ducts are thickened and nodular.

2. A section through the common bile duct of a mouse of the C_3H strain, which had received 50 μg of estradiol benzoate weekly for 218 days. ($\times$ 90.) The mucosa was thick and many gland-like processes extended to the serosa.

3. A section through the common bile duct of an untreated hybrid mouse (A X C_3H), killed at 273 days of age. $\times$ 90.

treated mice uniformly showed an extensive increase of the epithelial folds and gland-like processes which completely penetrated the thickened mucosa, the muscularis and frequently projected to the serosa (Fig. 2). The epithelium consisted of tall columnar cells which showed slight mitotic activity. In many ducts 2 types of cells were found, tall cells with an eosinophilic cytoplasm and cells with a clear cytoplasm. Evidence of cystic distension of the ducts, concretions or inflammatory processes were seldom found and never extensive. The lamina propria was histologically unchanged and the muscularis showed no hypertrophy, in fact, was difficult to discern in many hypertrophied ducts. The larger branches of the common duct were similarly involved but the intrahepatic ducts had not been affected.

Hypertrophy of the biliary ducts was observed in many mice of several different inbred strains (CB.A, C_3H, C_{57}, JK, A) and hybrids which have received estradiol dipropionate and estradiol benzoate in oily solution in amounts ranging from 16.6 to 50 μg weekly, estrone pellets, or stilbestrol (250 μg weekly). All animals were maintained on a diet of Purina Fox Chow and water. The hyperplasias occurred most frequently and were most extensive in mice receiving treatment for the longer periods. Most mice surviving treatment over 450 days showed lesions of the biliary ducts. In one series of experiments 58 of 76 mice (F_1 hybrids of CBA $\times$ C_{57} strains) receiving 16.6 μg of estradiol benzoate had moderately to extremely enlarged biliary ducts. Only 4 animals had ducts that were grossly normal. The incidence was lower in mice of groups which did tolerate the injections for the longer periods. Untreated mice (with the exception of 2 castrated females with adrenal tumors) and mice receiving testosterone propionate or sesame oil, have not shown a hypertrophy of the biliary ducts.

12046

Pulmonary Circulation Time in Man at Low Body Temperatures.

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Through the coöperation of Dr. Temple Fay, we have had an opportunity to observe a large number of patients during a period

of hypothermia, induced in accordance with his new methods designed to establish the effects of low temperatures upon inoperable malignant growths.[1] Since little is known about the effects of this procedure upon the various functions of the human organism, we have undertaken a study of the circulation rate as affected by reduction in the temperature of man to an extent sufficient to induce artificial hibernation.

Methods. Circulation time was determined by the cyanide method.[2] The overwhelming factor in the results obtained is undoubtedly the circulation time through the lungs. Each patient was first tested at complete rest at normal temperatures. For this test basal conditions were obtained either by voluntary coöperation, if possible, otherwise by the administration of evipal intravenously or avertin by rectum in dosage sufficient to produce narcosis. This narcosis was confirmed by the patient's failure to shiver (after the circulation time test) when cooled by cracked ice applied directly on the entire body surface (except the head) or by a blanket containing coils containing mixtures which did not freeze when held at low temperatures. In either case (voluntary coöperation or anesthesia), the patient had been at rest at least 15 minutes before the test.

During the period of low environmental temperature,[1] the patients were entirely quiet when tested, anesthesia being used if necessary to bring this about. Patients who had an anesthetic as in the control period were tested with an anesthetic in the period of hypothermia and those that were quiet without anesthesia in the first were tested without it in the second. Blood pressure and pulse rate were recorded during the 2 temperature periods.

Skin temperatures were determined just above (2 cm) the antecubital space by thermocouple. Rectal temperatures were also recorded by a recording resistance thermometer.

With every injection of cyanide all the precautions mentioned by Robb and Weiss[2] were observed. Three sites of injection were used, antecubital, jugular, and femoral veins. This last was selected as the best measure of flow along an intraäbdominal vein. Injections were made rapidly (0.5 second); a 2% solution of sodium cyanide was used. Volumes employed were 0.3 to 0.6 cc (6-12 mg).

Circulation time as measured by this method is the time required for the injected cyanide to pass from the site of injection to the carotid and aortic chemoreceptors, stimulation of which by the

[1] Smith, L. W., and Fay, T., *J. A. M. A.*, 1939, **113**, 653.

[2] Robb, G. P., and Weiss, S., *Am. Heart J.*, 1932, **8**, 650.

drug elicits an abrupt increase in respiration, usually in depth only, sometimes in rate and depth. This time was measured graphically by a pneumograph-tambour system recording on a kymograph and checked by a stopwatch.

Results. A. Circulation Time Between an Antecubital Vein and the Aortic-carotid Chemoreceptors. In a series of 14 patients, this interval was measured at normal temperatures under basal conditions (with or without anesthesia) and again in the cold. The interval between injection of sodium cyanide into the antecubital vein and its arrival at the arterial chemoreceptors, as indicated by the respiratory reaction, was prolonged from an average of 16.6 seconds (14 determinations) at normal body temperature to an average of 23.5 seconds (14 determinations) in the cold. The controls varied from 11.5 seconds to 25.9 seconds. The values in the low temperature phase varied from 17.8 seconds to 30.8 seconds. Statistical computation reveals a correlation between extent of prolongation of circulation time and extent of reduction of rectal temperature of +0.32, which is scarcely significant. (Table I.)

B. Circulation Time Between a Jugular Vein and Aortic-carotid Chemoreceptors. This was measured in 10 patients at normal temperatures and again in the same patients exposed to low temperatures. The interval between the injection and the respiratory reaction was increased from an average of 11.3 seconds (10 determinations) at normal temperatures to an average of 15.2 seconds (10 determinations) in the cold. For the controls at normal temperatures the upper and lower limits were respectively 18.0 and 8.8

TABLE I.
Antecubital

Case No.	Prolongation in circulation time (sec)	Fall in rectal temp. (°F)
1	13.5	13.8
2	15.0	9.0
3	10.4	10.3
4	2.5	9.6
5	13.5	14.6
6	15.4	3.8
7	3.6	8.8
8	9.4	12.2
9	3.0	11.6
10	1.8	14.6
11	1.1	13.6
12	8.2	11.1
13	6.1	10.1
14	1.2	14.1
	Correlation +0.32	

TABLE II.
Jugular.

Case No.	Prolongation in circulation time (sec)	Fall in rectal temp. (°F)	Fall in skin temp. (°F)	Changes in index of peripheral venous flow* (sec)
3	3.0	10.3	4.8	— 7.4
6	2.4	3.8	6.5	—13.0
7	1.0	8.8	6.0	— 2.6
8	0.8	12.2	14.5	— 8.6
9	0.3	11.6	9.0	— 2.7
10	2.8	14.6	10.0	+ 1.0
11	10.4	13.6	8.0	+ 9.3
12	6.5	11.1	5.0	— 1.1
13	1.9	10.1	4.0	— 4.2
14	5.2	14.1	3.0	+ 4.0
	Correlation +0.31		Correlation —0.20	

*Prolongation —; Decrease +.

seconds, for the cold 21.6 and 9.1 seconds. Here again there was little or no correlation between the extent to which circulation time was prolonged and the amount of reduction in rectal temperature (+.31, statistical computation, Table II). The jugular vein to arterial chemoreceptor time represents what Robb and Weiss have termed the crude pulmonary circulation time.[2] It is evident that although this interval is prolonged by cold, the indicated slowing in the pulmonary circulation is not proportional to the amount of cooling.

In 10 of these patients the index of peripheral venous velocity (antecubital to chemoreceptor minus jugular to chemoreceptor time) was determined. This measured the approximate velocity of venous blood flow in the arm. Here the magnitude of the change in circulation rate was not correlated with the extent of reduction in skin temperature (statistical computation). The relationship varied from 1.3 seconds *shortening* to 2.0 seconds *prolongation* per degree Fahrenheit reduction in skin temperature. (Correlation was –.20, Table II.)

C. Circulation Time Between a Femoral Vein and Carotid-aortic Chemoreceptors. The same methods in 10 patients as in A and B gave an increase (prolongation) in the average circulation time from 11.7 seconds (10 determinations) in the normal controls to 17.4 seconds (10 determinations) in the same cases exposed to low temperatures. Here, however, there was a statistically significant correlation (+0.81) between the amount of reduction in rectal temperature and the extent of the increase in circulation time (Table III).

TABLE III.
Femoral.

Case No.	Femoral circulation time Control	In cold	Prolongation in circulation time (sec)	Fall in rectal temperature (°F)	No. of tests
3	9.8	14.0	4.2	10.3	1
6	13.4	13.8	0.4	3.8	2
7	12.4	15.0	2.6	8.8	1
8	11.2	17.6	6.4	12.2	1
9	10.4	17.4	7.0	11.6	1
10	12.8	22.8	10.0	14.6	1
11	12.7	18.6	5.9	13.6	2
12	12.9	17.6	4.7	11.1	1
13	9.9	16.6	6.7	10.1	1
14	11.9	20.6	8.7	14.1	1
Avg	11.74	17.40	Correlation +0.81		

D. Effects of Shivering. In 2 subjects shivering was present because of insufficient depth of anesthesia during the stage of cooling. Both of these were also tested in the cold without shivering and at normal body temperature. In the first of these patients, shivering was quite pronounced. Circulation times from antecubital, jugular, and femoral veins to arterial chemoreceptors were all shorter than the corresponding times measured at normal temperatures. When the tests were repeated in the cold without shivering (*i. e.*, under deeper narcosis), the 3 circulation times were all prolonged over the control at normal temperatures.

In the second patient, shivering was only slight and was confined to the extremities. In this instance, circulation time from an antecubital vein was shorter than the control at normal temperature while the other two were increased but slightly. When retested in the cold under adequate narcosis without shivering, all 3 times were prolonged over the times recorded in controls at normal temperature.

E. Effect of Anesthesia. Three patients were used to test this factor. Examination proved them to have normal cardio-vascular systems. Circulation times were measured from the 3 sites of injection before and during evipal anesthesia. In all 3 cases the circulation times were shorter from each injection site under the anesthetic than at rest without it. Pulse rate was increased and blood pressure was lowered in all 3 cases during the narcosis.

It is probable that other types of anesthesia would produce somewhat different results, especially on blood pressure, but since evipal was used as the narcotic in most of our patients to whom any such drug was given, these observations show that the anesthetic *per se* was not a prominent factor.

F. Effects of Cooling on Pulse Rate and Blood Pressure. Pulse

rates were usually slower and blood pressure regularly lower in the cold than in the same patients at normal temperatures. In 2 instances in which pulse rate was faster in the cold, the circulation time was nevertheless prolonged.

Discussion. The overwhelming factor in the results obtained by the cyanide method must be represented by circulation through the lungs. Since pulmonary circulation time varies inversely with minute volume of the right heart and directly with capacity and resistance of the pulmonary circuit, measurement of pulmonary circulation time and its variations is a criterion of the resultant of these two factors acting simultaneously.[3, 4, 5] Our results, therefore justify the conclusion that when human patients are exposed to low enviromental temperatures by which their rectal temperatures are reduced to levels approaching 85°F, the balance between right heart minute volume and pulmonary capacity and resistance is shifted toward the latter.

A low environmental temperature reduces metabolism of brain as measured by smaller arterio-venous differences despite a slower blood flow.[6] If a similar reduction occurs in heart muscle under similar circumstances, the weaker cardiac contractions that would result could, by diminishing cardiac output, account for almost all of the prolonged circulation time as well as the slower pulse rate and lower blood pressure.

Patients in cold baths have a smaller cardiac output than controls.[7, 8] This suggests changes in right heart output as the largest factor to be considered. Although the dogs' heart will contract at temperatures as low as 77°F, the output near this lower extreme is apt to be reduced as the heart begins to fail. Our low temperatures may fall in the range of diminished cardiac output. Right heart failure with recovery has been confirmed clinically in one of these cases.[9]

Absence of correlation of extent of prolongation of antecubital-arterial chemoreceptor time with the amount of reduction of rectal temperature confirms the necessity of controlling arm temperatures

3 Stewart, G. N., *J. Physiol.*, 1894, **15**, 465.

4 Wiggers, C. J., *Physiol. Rev.*, 1921, **1**, 239.

5 Grollman, A., *The Cardiac Output of Man in Health and Disease*, C. C. Thomas, Springfield, Ill., 1932.

6 Fazekas, J. F., and Himwich, H. E., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 537.

7 Eismayer, G., and Czyrnick, W., *Z. Kreislaufforchg.*, 1934, **26**, 226.

8 Bornstein, A., Budelmann, G., and Ronnell, S., *Z. Klin. Med.*, 1931, **118**, 596.

9 McNair-Scott, T., personal communication.

when using the antecubital vein as a point of injection and the difficulty in interpretation of results obtained.[10]

A significant correlation of femoral-arterial chemoreceptor time with rectal temperature suggests that in the tissues which contribute blood to this long extent of intraäbdominal and intrathoracic vein, the volume of blood flow is reduced in direct proportion to the decrease in internal (rectal) body temperature, while in the case of the arm and head this is not true. It has been suggested that this correlation may be an expression of Van't Hoff's Law.[11, 12]

Summary. Pulmonary circulation time is prolonged in humans whose internal temperature is reduced toward 85°F when exposed to low environmental temperatures. It is suggested that diminished output of the right heart is a large factor. Correlations between fall in rectal temperature and prolongation in circulation time exist only when the femoral vein is the site of injection. Shivering shortens circulation time or minimizes prolongation due to hypothermia.

12047

Reflex Inhibition of Bile Flow and Intestinal Motility Mediated Through Decentralized Celiac Plexus.

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The celiac ganglia are anatomically and functionally related to the splanchnic nerves. The peripheral links in splanchnic efferent conduction pathways are made up of celiac ganglion cells. Splanchnic nerve components, however, are not the only nerve fibers which terminate in the celiac ganglia. In a recent experimental anatomical study, terminal branches of axons have been demonstrated in preparations of the celiac ganglia of the cat in which all the splanchnic nerve fibers which enter these ganglia had undergone degeneration, following bilateral section of the splanchnic nerves. Intact nerve fibers also have been demonstrated in sections of the distal segments of divided mesenteric nerves arising from the celiac plexus, after the fibers separated from their cells of origin had undergone degeneration.[1] These findings support the assumption that axons of

10 Stead, E. A., Jr., and Kunkel, F., *Am. J. Med. Sc.*, 1939, **198**, 49.

11 Ring, G. C., *Am. J. Physiol.*, 1939, **125**, 244.

12 Bruhn, J. M., *Am. J. Physiol.*, 1940, **129**, 322.

1 Kuntz, A., *J. Comp. Neur.*, 1938, **69**, 1.

enteric origin enter the celiac ganglia. If such fibers effect synaptic contacts with celiac ganglion cells, they might constitute the afferent limbs of reflex arcs with central connections in the celiac ganglia. The present investigation has been undertaken to test the hypothesis that reflex reactions can be mediated through the celiac plexus in the absence of intact nerve fiber connections with the central nervous system.

Methods. The experiments have been carried out on cats under nembutal anesthesia. The splanchnic and vagus nerves were divided bilaterally and the intestine transected in the lower portion of the jejunum. In one series of experiments, the common bile duct was cannulated and the drops of bile recorded. After the rate of flow was established, mesenteric nerves were stimulated either by means of a faradic current or distention of the ileum and colon. In another series, a balloon was placed in the proximal segment of the intestine and a kymographic record taken of the intestinal motility. While this segment was undergoing spontaneous contractions, mesenteric nerves to the distal segment of the intestine were stimulated either faradically or by means of distention of the ileum and colon.

Results. Inhibition of bile flow (Table I). In a series of control experiments carried out on cats with intact splanchnic and vagus nerves, distention of the ileum and colon and faradic stimulation of mesenteric nerves resulted in inhibition of the flow of bile. These

TABLE I.

Effects of Distention of Ileum and Colon and of Faradic Stimulation of Mesenteric Nerves on Rate of Bile Flow Before and After Bilateral Section of Splanchnic and Vagus Nerves.

	Control					After section of splanchnic and vagus nerves				
		Distention of ileum and colon		Faradic stimulation of mesenteric nerves			Distention of ileum and colon		Faradic stimulation of mesenteric nerves	
Cat No.	Bile flow drops per hr*	Flow drops per hr*	% change	Flow drops per hr*	% change	Bile flow drops per hr*	Flow drops per hr*	% change	Flow drops per hr*	% change
1	54.5			42.7	—21	80			40	—50
2	34.2			20	—41	28.5			13.3	—53
3	18.7			15	—19	27.9			7.8	—71
4	60	36.3	—39			42.7	25	—41	13.1	—71
5						42.7	30.7	—28	26	—39
6						32.4			25	—23
7						33.3			16	—52
8						29.2			18.7	—35
9						42.7	26	—40		
10						18.1	9.8	—45		

*Calculated on basis of five drop-intervals.

results are in general agreement with those of experiments carried out on dogs by Goldman and Ivy.[2] The same stimulation also resulted in inhibition of the flow of bile following bilateral section of the splanchnic and vagus nerves and transection of the jejunum.

Distention of the ileum and colon, by filling with air under moderate pressure, resulted in inhibition of the bile flow in all of 4 cats with decentralized celiac plexus and transected jejunum. The flow was reduced 28 to 45% during the periods of observation, or an average of 38.5%. Faradic stimulation of mesenteric nerves to the ileum resulted in inhibition of the bile flow in all of nine cats prepared in the same manner. The flow was reduced 23 to 71% during the periods of observation, or an average of 50%. A 1% solution of nicotine applied to the decentralized celiac ganglia effectively abolished the inhibitory response. In all of these experiments stimulation was maintained for 5 minutes. The period of observation covered the time interval required for the elimination of 5 drops of bile after the initiation of stimulation.

Inhibition of intestinal motility (Fig. 1). In the series of experiments in which the effects of distention of the ileum and colon and faradic stimulation of mesenteric nerves on the motility of the proximal segment of the intestine, following decentralization of the celiac plexus and transection of the intestine, were recorded kymographically, many trials yielded no positive results, *i. e.*, the stimulation resulted in no change in the kymographic record. In some experiments, the stimulation employed resulted in inhibition of the intestinal contractions. Rhythmic segmental contractions seemed to be inhibited more readily than peristaltic contractions. In the

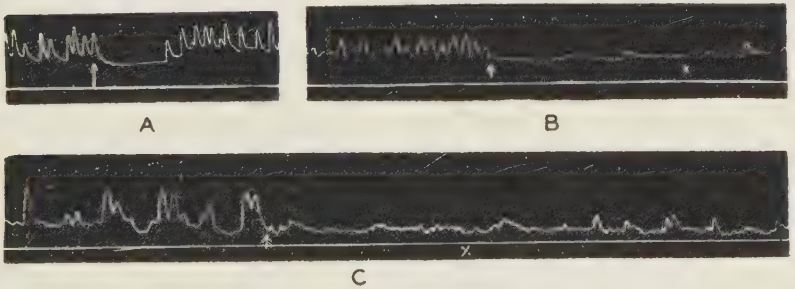

FIG. 1.

Kymographic records showing inhibition of motility in the proximal segment of the intestine following bilateral section of the splanchnic and vagus nerves and transection of the jejunum, elicited by (A) faradic stimulation of mesenteric nerves to the ileum and (B and C) distention of the ileum and colon. Initiation of stimulation is marked by an arrow; its discontinuance by X.

2 Goldman, L., and Ivy, A. C., *Ann. Surg.*, 1939, **110**, 755.

animals in which inhibition of intestinal motility was elicited, this could be accomplished only a few times. After inhibition had been elicited once or oftener, the same stimulation in additional trials usually resulted in no change in the kymographic record.

The record in Fig. 1A shows complete inhibition of rhythmic contractions in the proximal segment of the intestine for a brief interval, elicited by faradic stimulation of mesenteric nerves to the distal segment of the small intestine. In this instance contractions were resumed before stimulation of the mesenteric nerves ceased. The record in Fig. 1B shows inhibition of segmental contractions; the one in Fig. 1C, inhibition of peristaltic contractions, in the proximal segment of the intestine, elicited by distention of the distal segment, including ileum and colon, by filling with air under moderate pressure. In both instances, inhibition was nearly complete for several minutes, after which motility was resumed and gradually increased. The inhibitory effect of the stimulation remained apparent for several minutes after the 5-minute interval during which the distention was maintained.

Discussion. The inhibitory responses in the biliary system and the proximal segment of the intestine described in the present paper exhibit the essential properties of reflex reactions. They undoubtedly involve conduction pathways through the celiac plexus. They could not be influenced by centers in the central nervous system, since all connections of the celiac plexus with the central nervous system were interrupted. Complete division of the splanchnic and vagus nerves was verified in every animal used. They could not be mediated through the enteric plexuses, since the intestine was transected and the stimulation was applied distal to the section. The anatomical findings cited above support the assumption that the celiac ganglia may include reflex connections, but conclusive physiological data supporting this assumption have not been forthcoming. Warkentin and Ivy[3] have demonstrated that enterogastric regurgitation in response to acid irritation of a Thiry fistula of the duodenum in the dog may be mediated through the celiac plexus under certain experimental conditions in some cases. In their experiments, however, regurgitation was materially reduced after vagus section; consequently, they do not regard their findings as unequivocal evidence that the celiac ganglia constitute a true reflex center.

In view of the anatomical evidence that nerve fibers of enteric origin enter the celiac ganglia, the inhibitory responses described

[3] Warkentin, J., and Ivy, A. C., personal communication.

in the present paper in animals with the celiac plexus decentralized and the intestine transected, can be explained most satisfactorily as true reflex reactions. Abolition of these responses by application of a nicotine solution to the decentralized celiac ganglia also supports the assumption that the neural mechanisms employed include synaptic connections in these ganglia.

By what mechanism the flow of bile is inhibited has not been determined. Vasomotor reactions in the liver undoubtedly play a rôle in this response. The formation of bile, according to Tanturi and Ivy,[4] is influenced by intrahepatic vascular pressure. In their experiments, stimulation of the sympathetic nerves to the liver resulted in decreased bile formation, probably due to vascular or mechanical changes. True inhibitory secretory nerves to the liver have not been demonstrated. Inhibition of intestinal motility, as demonstrated in our experiments, probably is effected by impulses reaching the intestinal musculature through its sympathetic innervation

12048

Effect of Sulfanilamide and Sulfapyridine on the Avian Malarias.*

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The effect of sulfanilamide and sulfapyridine on some of the bacterial infections has been so striking that these drugs have been tried on many others, including some of the malarias, both of man and of animals. The results obtained have been of considerable interest, and not the less so because they have varied a great deal with the species. Coggeshall,[1] for example, found that sulfanilamide was effective in eradicating *Plasmodium knowlesi* infection in monkeys, but was apparently without any action on *Plasmodium inui* in the same host. He suggested that the difference might be due to the much more rapid metabolism of the former species. This seems probable in view of the generally accepted idea that sulfanilamide is effective because oxidation products are formed from it which destroy catalase, and hence interfere with the metabolic activities of the cell.

[4] Tanturi, C. A., and Ivy, A. C., *Am. J. Physiol.*, 1938, **121**, 61.

* Aided by a grant-in-aid from the American Philosophical Society.

[1] Coggeshall, L. T., *J. Exp. Med.*, 1940, **71**, 13.

Since the malaria parasites are present in the blood stream they are presumably more exposed to this effect than if they were segregated somewhere in the tissues.

Against the malaria parasites of man sulfanilamide and its derivatives appear not to be of great value, although the reports are conflicting, and there seems to be but one report of its use in avian malaria. Coggeshall[2] states that when administered to chicks infected with *Plasmodium lophuræ* and canaries infected with *Plasmodium cathemerium* it was without noticeable influence. It has also been used on *Hemoproteus columbæ*, but failed to exert any effect (Durand and Villain[3]).

Since it is well-recognized that the various species of malaria plasmodia react quite differently to even the same drug, trial of sulfapyridine and sulfanilamide on some of the other species of avian malaria has been undertaken. So far all the tests have been made on blood-induced infections because such infections are more easily produced in the numbers required for extensive experimenting, and also because mosquitoes have not been available. And in the case of one of the species used, *Plasmodium nucleophilum*, nothing is yet known as to the natural vector.

Up to the present, we have used these drugs against 3 species of avian malaria, *Plasmodium circumflexum* (Strain E; Manwell and Goldstein[4]), *relictum* var. *matutinum*, and *nucleophilum*. The second of these 3 species was isolated from an English sparrow in December, 1938, and the third is the original type strain, isolated from a catbird in April, 1934. All experiments were carried out with female canaries.

Because of the relatively low solubility of the drugs both the sulfanilamide (Prontylin, Winthrop) and the sulfapyridine (furnished through the courtesy of E. R. Squibb and Sons) were given in suspension. Treatments were given daily (the dosage being 0.02 g in 100 mg of physiological saline) by intraperitoneal or intramuscular injection. This amount represented about 1/5 the M.L.D. The duration of treatment varied considerably, particularly in the experiments involving sulfapyridine and *Plasmodium circumflexum*, because it was desired to evaluate the effects of the drugs as accurately as possible. Details are given in Table I.

In summary, it may be said that only one of the species was affected by either drug. This was *Plasmodium circumflexum*, which

2 Coggeshall, L. T., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 768.

3 Durand, P., and Villain, *Arch. Inst. Pasteur de Tunis*, 1939, **28**, 94.

4 Manwell, R. D., and Goldstein, F., *Am. J. Hyg.*, 1939, **30**, 115.

TABLE I.
Effect of Sulfapyridine and Sulfanilamide on Three Species of Avian Malaria.*

Species	Treatment: When given	Treatment: Duration, days	Sulfapyridine: Exp. cases	Sulfapyridine: Controls	Sulfapyridine: Effect†	Sulfanilamide: Exp. cases	Sulfanilamide: Controls	Sulfanilamide: Effect
Plasmodium circumflexum (Strain E)	B + I	13-26‡	8	5	+++	10	7	—
	I	9	6	5	++	0	—	—
	A	5	6	5	+	0	—	—
Plasmodium var. matutinum	B + I	11-17‡	6	3	—	6	4	—
Plasmodium nucleophilum	B + I	12	6	2	—	4	2	—
Totals			32	20§		20	13§	

*Abbreviations used as follows:
B = before inoculation
I = during incubation period
A = during acute stage
Drugs given by intraperitoneal or intramuscular injection.
†This is indicated by plus signs which have the following meaning:
+++ no parasites or very few, but subinoculation showed that no birds were sterilized.
++ a few parasites seen in some cases, but duration of infection short.
+ mild infections, but heavier than those indicated with a double plus.
‡Birds were matched with controls and treated until parasites began to diminish in latter. Hence some were treated for longer periods than others.
§The total number of controls was 22; totals given are larger because some birds served as controls in series in which some birds received one drug and some the other.

was markedly susceptible to sulfapyridine. This drug was capable of aborting the appearance of parasites in the peripheral blood, and of causing their rapid disappearance after the infection becomes patent. But it is less effective than plasmochin, atebrine, or quinine.

Visible changes in the parasites were noticed only in this one species, and were especially evident in the older schizonts. These forms no longer stained normally, and the division process also seemed abnormal.

It is interesting that *Plasmodium relictum* var. *matutinum*, which is a relatively large and rapidly reproducing species (and hence of presumably high metabolic rate), appears to be entirely unaffected by either drug. It is also noteworthy that *nucleophilum* was not influenced, although it is one of the small species, several of which have previously been found especially susceptible to other antimalarial drugs. (Manwell,[5] Manwell and Haring[6].)

[5] Manwell, R. D., *Am. J. Trop. Med.*, 1932, **12**, 123; *Ibid.*, PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 198.

[6] Manwell, R. D., and Haring, Ann T., *Riv. d. Parasit.*, 1938, **2**, 209.